

## ERRATUM.

Article 7032, fourth sentence should read, "The use of autogenous staphylococcus vaccines and the usual sanitary precautions had during the previous year failed to keep the disease in check. . . ."

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## Missouri Section.

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7107 P\*

### Hyperglycemia Produced by Extracts of Normal Urine.

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Application of the tungstic acid precipitation<sup>1</sup> to the concentration of the active principles of urine led to the discovery that an extract of the urine of men produces a distinct hyperglycemia in fed rabbits. A review of the literature revealed only the papers of Böhm<sup>2</sup> and Eidelsberg,<sup>3</sup> who reported that the intravenous injection of gonadotropic extracts of pregnancy urine produced a marked hyperglycemia. Dingemanse and Kober<sup>4</sup> and Houssay<sup>5</sup> have added information on this point.

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\* P represents a preliminary, C a complete manuscript.

<sup>1</sup> Katzman, P. A., and Doisy, E. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1188.

<sup>2</sup> Böhm, F., *Z. f. des. ges. exp. Med.*, 1932, **84**, 689.

<sup>3</sup> Eidelsberg, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 959.

<sup>4</sup> Dingemanse, E., and Kober, S., *Endocrinol.*, 1933, **17**, 149.

<sup>5</sup> Houssay, B. A., and Biasotti, A., *Compt. rend. Soc. Biol.*, 1933, **113**, 469.

Of course, it is possible that the hyperglycemic action of extracts of urine is due to the diabetogenic substance of the anterior lobe of the hypophysis, (Houssay,<sup>6</sup> Evans,<sup>7</sup> and others) but our work thus far does not permit us to draw such conclusions.

*Preparation of Extracts.* The substance has been concentrated by the usual methods<sup>1, 8</sup> employed in this laboratory for the preparation of the gonadotropic factor of pregnancy urine. The benzoic acid adsorption process described by Katzman and Doisy<sup>8</sup> gave active extracts of this factor from normal male urine. In this case the acetone insoluble residue, after removal of the benzoic acid, was leached with dilute alkali (pH 8-9) instead of water. Table I presents the data obtained with this type of extract.

TABLE I.  
Adsorption Methods.

| Method       | Preparation No. | Urine equivalent of extract injected<br>Liters | Initial blood sugar<br>Mg % | Maximum blood sugar<br>Mg % | % Increase | Time elapsed before maximum value<br>Hr. |
|--------------|-----------------|------------------------------------------------|-----------------------------|-----------------------------|------------|------------------------------------------|
| Benzoic Acid | MB 1            | 12                                             | 143                         | >300                        | >110       | 4                                        |
|              | MB 2            | 4                                              | 148                         | 166                         | 12         | 6                                        |
|              | MB 3            | 5                                              | 117                         | 135                         | 15         | 4½                                       |
|              | MB 4            | 10                                             | 121                         | 150                         | 24         | 5                                        |
|              | MB 5            | 7                                              | 145                         | >300                        | >107       | 3                                        |
|              | MB 6            | 7                                              | 136                         | 200                         | 47         | 3½                                       |
| Kieselguhr   | MA 1A           | 6                                              | 155                         | 153                         | —          | 5                                        |
|              | MA 1B           | 9                                              | 163                         | 272                         | 66         | 4½                                       |
| Alumina Gel  | ML 1            | 2                                              | 143                         | 150                         | —          | 4                                        |
| Kaolin       | MK 1            | 7.6                                            | 167                         | >300                        | >100       | 3½                                       |
|              | MK 1            | 3.8                                            | 157                         | 212                         | 35         | 4                                        |
| Charcoal     | MC 1            | 3                                              | 135                         | 126                         | —          | 3½                                       |

Tungstic acid precipitation (Katzman and Doisy<sup>1</sup>) was also effective in concentrating the active factor. Clear and more potent extracts are obtained by liberating the active material from the tungstic acid precipitate by means of an alkaloid instead of the sodium hydroxide (Table II). In this procedure the acetone washed tungstic acid precipitate is stirred with an excess of an aqueous suspension of brucine and centrifuged. The brucine is removed by extracting the alkaline solution with chloroform or by precipitation with acetone. Other alkaloids can probably be used in place of the brucine.

<sup>6</sup> Houssay, B. A., and Biasotti, A., *Compt. rend. Soc. Biol.*, 1930, **104**, 407; *Ibid.*, 1930, **105**, 121.

<sup>7</sup> Evans, H. M., Meyer, K., Simpson, M. E., and Reichert, F. L., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 857.

<sup>8</sup> Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

TABLE II.  
Tungstic Acid Method.

| Preparation No. | Urine equivalent of extract injected Liters | Initial blood sugar Mg % | Maximum blood sugar Mg % | % Increase | Time elapsed before maximum value Hrs. |
|-----------------|---------------------------------------------|--------------------------|--------------------------|------------|----------------------------------------|
| T76b            | 5-6                                         | 137                      | 242                      | 80         | —                                      |
| T103            | 8                                           | 153                      | 245                      | 56         | 3¼                                     |
| MR 1            | 2½                                          | 186                      | 311                      | 67         | 3                                      |
| MR 2            | 1.6                                         | 139                      | 230                      | 65         | —                                      |
| MR 3            | 2                                           | 129                      | 182                      | 41         | 6                                      |
| MR 4*           | 4                                           | 122                      | 150                      | 23         | 3                                      |
| MR 5*           | 4                                           | 115                      | 131                      | 14         | 5                                      |
| MR 6*           | 8½                                          | 123                      | 146                      | 20         | 4                                      |
| MR 7b           | 14½                                         | 127                      | 249                      | 96         | 4                                      |
| MR 10           | 16                                          | 137                      | 222                      | 62         | 5                                      |
| MR 11           | 2                                           | 168                      | 208                      | 24         | 4½                                     |
| MRa 17†         | 3                                           | 165                      | 225                      | 37         | 3½                                     |
| MRa 19†         | 3                                           | 161                      | 251                      | 56         | 3½                                     |

\*Assayed on animals starved 14 to 18 hours.

†Brucine used for extraction.

Considerable purification and concentration of our extracts can be obtained by acetone precipitation. Addition of 4-6 volumes of acetone does not completely precipitate this factor as the acetone solution is usually somewhat active.

From observations of our crude extracts it appears that the activity is lost on standing in the cold room and on treatment with acid, alkali or heat. The substance is soluble in water, aqueous methyl or ethyl alcohol or acetone and insoluble in ether, absolute alcohol, chloroform and strong acetone.

The extracts were tested for hyperglycemic action by following the blood sugar of rabbits for 5 or 6 hours after subcutaneous injection. Since animals fasted as for insulin assays showed but slight increases in blood sugar, food was not withdrawn until shortly before starting the experiment. This accounts for the high preliminary blood sugar values.

## Effect of Dinitrophenol on Oxidation of Tissues.

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The marked acceleration of metabolism of animals injected with small quantities of dinitro  $\alpha$  naphthol reported by Heymans and Bouchaert,<sup>1</sup> Van Uytvanck,<sup>2</sup> and Euler,<sup>3</sup> and a similar action of dinitrophenol observed by Magne, Mayer and Plantefol<sup>4</sup> and by Tainter and Cutting,<sup>5</sup> make it of interest to learn whether these substances affect the rate of respiration of isolated tissues and cell suspensions *in vitro*. If that be the case, it may be hoped that a study of the action of these substances will aid in elucidating the mechanism of cellular oxidation and metabolism. We have studied the effect of 2-4 dinitrophenol on the respiration and anaerobic fermentation of yeast and of frog tissues, using Warburg vessels. With muscle, lactic acid was also determined chemically.

Dinitrophenol added to the solutions in which the tissues or yeast are suspended, in concentrations of the same order as perhaps exists in the tissue fluids after administration of toxic or even large therapeutic doses to man or animals, causes a marked increase of respiration of tissues so far examined. With increasing concentrations, the effect rises to a maximum beyond which the rate of respiration is progressively decreased. The optimum for frog tissues at 25° pH 7.5 is 0.5 mg. % and for yeast at 30° pH 4.5 0.36 mg. %.

The effect is most marked with frog muscle, the resting respiration of which is raised about 8 times above the normal, or to a level somewhat above that observed in similar muscles after maximum stimulation or even hashing. The marked increase caused by dinitrophenol is not accompanied by twitching, and the muscle remains irritable for at least 3 hours. A marked acceleration of anaerobic lactate production is caused by dinitrophenol, and reaches levels

<sup>1</sup> Heymans, C., and Bouchaert, J. J., *Comp. rend. Soc. Biol.*, 1928, **99**, 636; *Arch. Intern. Pharm. et de Ther.*, 1928, **35**, 63.

<sup>2</sup> Van Uytvanck, P., *Comp. rend. Soc. Biol.*, 1930, **103**, 29; *Arch. Intern. Pharm. et de Ther.*, 1931, **41**, 160.

<sup>3</sup> Von Euler, U. S., *Comp. rend. Soc. Biol.*, 1931, **108**, 249; *Arch. Intern. Pharm. et de Ther.*, 1932, **43**, 67.

<sup>4</sup> Magne, H., Mayer, L., and Plantefol, L., *Ann. de physiol.*, 1932, **8**, 1.

<sup>5</sup> Tainter, M. L., and Cutting, W. C., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 1268.

observed with hashed muscle. The controlling factor of lactic acid production present in normal muscle appears to be removed by the action of dinitrophenol and the process of glycolysis proceeds at nearly the maximum rate. In normal muscle at rest there is no aerobic glycolysis, but in the treated muscles there is an accumulation of lactic acid in spite of the high  $O_2$  consumption. A part of the acceleration in  $O_2$  consumption may therefore be explained by the increase in lactic acid.

With kidney an increase in  $O_2$  consumption of 75% was found, with liver 30% and with nerve 45%.

Dinitrophenol causes an acceleration of 50% in the rate of the oxygen consumption by yeast with glucose as substrate. Chemical analysis of the glucose lost during respiration shows an accelerated utilization. The oxygen consumed by normal yeast accounts for the complete oxidation of 33 to 40% of the glucose which disappears, while in yeast treated with dinitrophenol 70 to 75% of the glucose lost is accounted for by the  $O_2$  consumed. This suggests the inhibition of some other process by which yeast normally disposes of a part of the glucose consumed.

While the rate of anaerobic fermentation by yeast is unaffected (Cutting and Tainter<sup>6</sup>), the total  $CO_2$  produced is increased by dinitrophenol. Instead of the normal 75 to 80% of the theoretical  $CO_2$  from a given amount of sugar, 85 to 90% is formed by treated yeast. Aerobic fermentation, low in normal yeast, is somewhat accelerated in treated yeast, and the rapid oxidation rate continues after the glucose has disappeared; the products of oxidation are the fuel, since the yeast finally returns to the low rate for normal yeast. Dinitrophenol has only slight effect on the respiration of yeast in the absence of added substrate. When the fermentation and oxidation of glucose by yeast is inhibited by iodo-acetate, the addition of dinitrophenol causes no rise in respiration.

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<sup>6</sup> Cutting, W. C., and Tainter, M. L., *J. Pharm. and Exp. Ther.*, 1933, **48**, 410.

## Direction of Flow of Nasal Mucus.

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St. Louis.*

Information concerning patterns of ciliary movement in the nose of man and monkey is already available, but in order to learn the significance of the direction of mucous flow in various regions of the nose and its possible relation to physiological and anatomical factors, experiments were performed on numerous mammals, selected to include a wide range in size, the mouse, rat, rabbit, opossum, cat, sheep and cow.<sup>†</sup> The animals were decapitated and the lateral nasal wall exposed by cutting near the median line. Two methods were employed which doubly insured accuracy; one, the application of carbon particles to the mucous surface, is well known; and the second, which leaves the mucous layer undisturbed and permits the moving cilia themselves to be observed *in situ*, has recently been described.<sup>1</sup>

It was found, as a result of these experiments, that the mucous flow in the upper half to two-thirds of the lateral nasal wall including the middle meatus, is toward the anterior end of the nose in all the animals mentioned. Only the material collected from the ethmoid conchae and those regions adjacent to the floor arrives in the nasopharynx. The material derived from the ostium of the maxillary sinus spreads, fan-like, to be distributed toward the anterior end, the floor, and the nasopharynx.

Evidently the direction of ciliary beat is adapted to protect the non-vibratile olfactory epithelium from accumulation of mucus along its border. This should be taken into account when fluids, bacterial suspensions, and viruses are experimentally introduced into the nose with the expectation that they will pass through the cribriform plate as a portal of entry into the central nervous system.

The entire mucous sheet in man and monkey ultimately reaches the nasopharynx and none passes out of the anterior nares, which is

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\* Aided by grants from the National Research Council and the Chemical Foundation and an appropriation from a grant from the Rockefeller Foundation to Washington University for research in science.

† We are indebted to the Independent Packing Company of St. Louis for the material and space to study the noses of freshly killed sheep and cows.

<sup>1</sup> Lucas, A. M., *Arch. Otolaryngol.*, 1933, **18**, 516.

quite different from the condition found in the animals described and in the latter the region of greatest ciliary activity is in the upper part of the nose and the flow of mucus in the lower half and along the floor is very sluggish. This also is exactly opposite to the condition found in man and monkey.

The direction of air currents was then determined for the cat by plotting out the distribution of inhaled carbon and it was found that it differed to an equal degree from the plan of air currents as marked out in man by Paulsen.<sup>2</sup> Therefore it was concluded that the direction of air currents through the mammalian nose is a factor in the development of the pattern of mucous flow and that the two are inversely correlated.

## 7110 P

### Histology of the Ovary of Hypophysectomized Rats Treated with Urinary Hebin.

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The treatment of hypophysectomized rats with hebin, the gonad stimulating substance from human pregnancy urine, indicates that the response is conditioned by the time intervening between hypophysectomy and the beginning of hebin administration and by the influence of hebin administration, immediately after hypophysectomy, upon a later treatment with hebin, after an intervening period of rest.

Female albino rats, weighing about 120 gm. when completely hypophysectomized by Dr. Smith's method,\* showed the usual ovarian changes with atresia of follicles, almost complete absence of follicular development, granulosa atrophy, interstitial tissue hyperplasia and production of wheel cells described by Selye *et al.*<sup>1</sup>

In all cases one ovary, removed at some crucial period in the experiment, was compared histologically with the second ovary, removed at the end of the experiment.

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<sup>2</sup> Paulsen, E., *Sitz. d. k. Akad. d. Wiss., Wien., Math-naturen*, 1882, **85**, 352.

\* The writer wishes to express his gratitude to Dr. Smith for explaining some of the details of the operation and to Dr. H. B. Van Dyke for demonstrating the operation.

<sup>1</sup> Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinology*, 1933, **17**, 494.

Urinary hebin† failed to inhibit ovarian degeneration or cause repair in 16 animals treated shortly after hypophysectomy. Serial sections from 5 of the animals showed that the ovary was converted into a lutein-like mass in which it was difficult to distinguish the individual component parts. Occasional small and medium sized follicles were found. The origin of the lutein is apparently from diverse kinds of cells but primarily from theca. The production of new corpora lutea reported by Leonard and Smith<sup>3</sup> is somewhat uncertain in our animals.

Other animals similarly treated and then allowed to rest for 12 to 20 days showed a return to the involuntary state as if no hebin had been administered. The previously luteinized theca changed over to the wheel cell type. A few luteinized follicles persisted but did not exceed in number those found in the ovaries of untreated hypophysectomized rats of comparable periods after hypophysectomy.

In 5 animals the terminal response to the injection of hebin after a 6 to 10 weeks interval after hypophysectomy was quite similar to the response to hebin administration immediately after hypophysectomy. The difference was that theca luteinization in delayed hebin administration occurred as a transformation of previously formed wheel cells into lutein cells while no such intermediate wheel cell formation occurred when hebin was injected immediately after hypophysectomy.

Twenty-one animals previously treated with hebin shortly after hypophysectomy and then allowed to rest for 12 to 20 days and then reinjected with large doses of hebin gave quite different reactions. Large doses did not produce any obvious change in the ovary. Thecal wheel cells persisted and in some cases seemed to increase in number with no increased luteinization of the ovary. The hebin used was active as checked in other experiments. Cornified vaginal smears were always produced after all hebin injections.

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† Prepared by the method of Katzman and Doisy.<sup>2</sup>

<sup>2</sup> Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

<sup>3</sup> Leonard, Samuel L., and Smith, Philip E., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 780.

## Minnesota Section.

*University of Minnesota, November 15, 1933.*

7111 C

### Effect of Previous Ileosigmoidostomy on Survival Period of Dogs with Low Ileal Obstruction.

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*Introduction.* A correlation of the survival period with the level of simple obstruction has been observed by many investigators. It is well established that dogs with obstruction of the upper jejunum will survive 2-4 days (average), of the lower ileum, 7-8 days (average); when the site of obstruction is the distal colon, the survival period may be as long as 50 days. Length of life thus varies directly with the distance of the site of the obstruction from the pylorus. The principal cause of the rapid death which follows high obstruction is due to changes in blood chemistry incident to dehydration and loss of gastro-intestinal secretions. The cause of death in low ileal obstructions is not so well understood. Elman and Hartmann<sup>1</sup> are of the opinion that in low ileal obstruction, the cause of death is not due to the above factors.

Wangensteen and Leven<sup>2</sup> say that saline does not protect animals with ileal or colonic obstructions. The survival period of dogs with complete ileal obstruction is little greater with subcutaneous saline than without. Carlson's<sup>3</sup> series of dogs with ileal obstruction (given saline) lived 2-14 days.

*Procedure and Method.* In an attempt to correlate the cause of death and survival period of ileal obstruction with the function of the bowel, the following experiments were undertaken. Ileosigmoidostomy was performed in 7 dogs, short circuiting almost the entire colon, in an effort to adapt the ileum to the function of the colon.

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<sup>1</sup> *Surg. Gynec. and Obst.*, 1931, **53**, 307.

<sup>2</sup> *Arch. Surg.*, 1931, **22**, 658.

<sup>3</sup> *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 421.

These dogs developed a transient diarrhea of 2 or 3 weeks' duration, which finally subsided with a return to previous body weight and normal bowel habits. After varying periods of time (2-8½ months) the dogs were operated upon again, the anastomosis taken down and obstruction of the terminal ileum produced in the usual manner (inversion of ends). Two of the obstructed animals were allowed to go without saline. The remainder received daily 500 cc. of normal saline subcutaneously. The blood chemistries (non-protein nitrogen, urea and chlorides) were determined every other day.

*Results.* Two dogs (without benefit of saline) lived 8 and 10 days respectively and lost approximately 17% body weight before death. They had had ileocolostomy only 2 months previous to obstruction. Of the 5 remaining animals (with benefit of saline) 2 lived 10 and 12 days losing 7.0 and 14.8% of body weight respectively. The last 3 dogs survived for 25, 31, and 34 days with a loss of from 28 to 40% of body weight before death. All showed a moderate fall of blood chlorides (only one below 300 mg.) and a gradual elevation of non-protein nitrogen and urea. The chemistry changes were most marked in the animals with the shortest survival periods. Five of the dogs died of the obstruction *per se*; one developed a volvulus above the site of obstruction, and the other a localized abscess from perforation of colon at the site of previous anastomosis.

Progressive distension was a prominent feature, especially in those dogs surviving 25, 31, and 34 days. In these, the bowel just above the site of the obstruction measured 8-10 cm. in diameter, about 4 times the normal. Gross areas of congestion and hemorrhagic changes in the mucosa were present on the antimesenteric border of the bowel. Microscopically these areas showed hemorrhagic necrosis of the mucosa. In one, Dog No. 70, several small areas of mucosal ulceration were evident.

*Discussion.* Ileosigmoidostomy was performed in 7 dogs to alter the function of the terminal ileum by adapting it to conditions of the colon, *i. e.*, (1) stasis, (2) absorption of water, and (3) bacterial flora. After 2-8½ months simple ileal obstruction was produced at the site of the anastomosis. Three of the dogs survived for 25, 31, and 34 days. This survival period is considerably longer than that of dogs with similar low ileal obstruction. A loss of 28 to 40% of body weight occurred in these 3 dogs before death. Death was due largely to starvation. Ileal obstruction on these dogs simulated large bowel obstruction, *i. e.*, distension, loss of weight, starvation, and increased survival period. This can best be explained as follows:

SUMMARY: ILEOSIGMOIDOSTOMY SERIES

| Dog | Duration |      | Survival period<br>after obstruction<br>saline<br>given | Weight<br>loss<br>% | Chemistry limits  |             | Chemistry at death | Pathology                                                                                                               |
|-----|----------|------|---------------------------------------------------------|---------------------|-------------------|-------------|--------------------|-------------------------------------------------------------------------------------------------------------------------|
|     | mo.      | days |                                                         |                     |                   |             |                    |                                                                                                                         |
| 37  | 2        | 8    | No                                                      | 17.6                | Cl.<br>U.         | 412<br>305  | 412<br>35.         | Distension and ruber of bowel wall                                                                                      |
| 29  | 2        | 10   | No                                                      | 17.                 | Cl.<br>U.         | 305<br>33.  | 305<br>33.         | Abscess about colon closure                                                                                             |
| 45  | 3½       | 12   | Yes                                                     | 14.8                | NPN<br>Cl.<br>U.  | 264<br>18.  | 65.<br>107.0       | 65.<br>Volvulus, segment of small bowel<br>with strangulation                                                           |
| 79  | 6        | 10   | Yes                                                     | 7.                  | Cl.<br>U.         | 379<br>150. | 379<br>50.         | 37.2<br>Hemorrhagic necrosis, antimesen-<br>teric border                                                                |
| 71  | 3½       | 25   | Yes                                                     | 23.                 | Cl.<br>U.         | 330<br>21.4 | 346<br>11.5        | 80.<br>Hemorrhagic areas of necrosis,<br>antimesenteric border.                                                         |
| 70  | 8        | 34   | Yes                                                     | 29.                 | NPN<br>Cl.        | 313         | 47.<br>22.5        | 22.5<br>Closed loop formed by adhesions;<br>hemorrhagic necrosis, antimesen-<br>teric border; ulceration of mu-<br>cosa |
| 74  | 8½       | 31   | Yes                                                     | 40.                 | NPN<br>Cl.<br>NPN | 363<br>40.  | 478<br>30.         | 37.<br>Hemorrhagic necrosis, antimesen-<br>teric border                                                                 |

The ileum after ileosigmoidostomy gradually accommodates itself to conditions of the colon, *i. e.*, (1) stasis; (2) greater bacterial flora; and (3) takes over its function of absorption of water. Upon the establishment of the obstruction, these functions which would then be lost to the organism because of exclusion of the colon (below obstruction) are preserved.

*Conclusions.* 1. Preliminary ileosigmoidostomy results in increased survival period following establishment of low ileal obstruction. 2. Adaptation of the ileum to conditions of the colon preliminary to obstruction results in increase of functional length with subsequent increased survival period. 3. The lethal factors in low ileal obstructions, although not well understood are apparently dependent upon some peculiarity or alteration of function of the bowel above the level of obstruction.

## Peiping Section.

*Peiping Union Medical College, October 25, 1933.*

7112 P

### Production of Ovulation by Anterior Lobe of Pituitary of Thyroidectomized Rabbit.

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In this study 14 litters of albino rabbits of homogeneous stock have so far been used. The control animals were all littermates except in 2 instances. The rabbits used for testing the quantity of the principle causing ovulation were albino rabbits purchased locally.

One hundred and thirty-nine to 333 days elapsed between thyroidectomy and death. At the time of killing the anterior pituitary lobes of the successfully operated animals (16) and their controls (14) were carefully dissected under a binocular magnifier and weighed to  $\pm 0.02$  mg. by means of a torsion balance. A careful search was made for thyroid tissue grossly; material which appeared possibly to be thyroid tissue was examined histologically. In 3 animals appearing to suffer from thyroid deficiency small amounts of thyroid were found. The individual anterior pituitary lobes were thoroughly ground in an agate mortar and suspended in 5.0 cc. of 0.9% NaCl solution. A part of such a suspension was then injected intravenously into one or more normal adult rabbits which were killed for examination of the ovaries about 24 hours later.

Among 10 litters in which thyroidectomy appeared to be complete and in which littermate controls were available, the mean body weights and standard deviations were: thyroidectomized,  $1.57 \pm 0.217$  kg.; control,  $2.49 \pm 0.380$  kg. The mean weights of the anterior lobes of these 2 groups were: thyroidectomized,  $34.0 \pm 5.41$  mg.; control,  $24.69 \pm 8.11$  mg. Statistically therefore the body-

weights and weights of anterior lobes were altered significantly by thyroidectomy.

The doses of fresh anterior lobe tissue necessary to cause ovulation in at least part of a group were found to be larger than those Wolfe and Cleveland used.<sup>1</sup> In recipient animals weighing about 3 kg., 5 mg. of fresh anterior lobe per kg. were required; in smaller animals (about 2 kg.) it was necessary to inject 7.5 to 10.0 mg. per kg. In using the anterior lobes of an operated and a control animal we usually administered doses proportional to the anterior lobe weights to recipient animals of practically identical weights. Our results indicate that the concentration of the hormone causing ovulation was reduced in the pituitary of the thyroidectomized animal. The absolute amount of the hormone present was certainly not increased and may have been reduced inasmuch as ovulation occasionally occurred only in the test animal receiving anterior lobe suspension from a control donor; moreover anterior lobe suspension from a control animal always caused ovulation, if a similar suspension from a thyroidectomized animal had a similar effect.

## 7113 P

### Fate of Bronchial Ligatures.

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It is well known that non-absorbable ligatures and sutures applied to some tubular structures in the body tend to migrate. The special mode of migration of such material in the intestine, common bile duct, Fallopian tubes, and large arteries has been determined in detail. The present work concerns the behavior of ligatures applied to a tertiary bronchus, and the resultant effect upon the bronchial continuity, in dogs.

The first branch (about 4 mm. in diameter) of the stem bronchus of the right lower lobe was exposed, and ligated with silk. In 9 dogs, it was singly ligated; in 8 other dogs it was ligated at 2 points and divided between the ligatures. After postoperative periods varying from 3 days to 6 months, the animals were killed and the region of ligation was examined grossly and microscopically (using serial microscopic sections).

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<sup>1</sup> Wolfe and Cleveland, *Anat. Rec.*, 1931, **51**, 213.

In every case of single ligation, before the 7th day, the thread cut through the bronchial wall at one point. The process continued, more or less gradually, until the ligature became entirely free and lay in the lumen. Almost as fast as the ligature cut through, the resultant circular ulcer became filled with granulations and covered with bronchial epithelium. At 6 months, the ligature had disappeared, presumably to the outside, the bronchial lumen was patent, and the walls were healed. There was little or no stricture. The mucosa was normal, but under it was a microscopic circumferential scar.

In every case of double ligation, the bronchus remained obstructed. The proximal stump (the one next to the stem bronchus) healed without any interruption; but within 14 days a small ulcer developed in the mucosa of the distal bronchial segment, at a point just over the distal ligature, and it stayed open until both ligatures and nearly the whole mass of pinched off cartilages had gradually worked their way into the lumen of the distal bronchial segment. At 6 months, the ulcer was healed and the fragments of silk thread were lying in the lumen distal to the site of bronchial interruption. The portion of the pinched off cartilages which failed to migrate underwent absorption *in situ*.

In both types of ligation, as long as the bronchus remained blocked, the distal lumen was greatly distended with air, mucus, leukocytes, and tissue debris. The parenchyma of the obstructed lobule was always air-containing.

The reason for the difference in the resultant effect upon bronchial continuity, which the 2 types of ligation exhibited, is not apparent. It corresponds with the situation in arterial ligation, where one tie applied "in continuity" usually fails to interrupt the vessel permanently but 2 ties with division between produces permanent interruption.<sup>1</sup> The cause for the migration of the ligatures and pinched off cartilages into the lumen distal to the site of ligation, rather than into the lumen on the proximal side, in the cases with double ligation, was apparently the fact that the lumen of the distal side only was distended, so that the bronchial wall on that side alone pressed against the ligated masses. The continued inflation of the obstructed lobules was obviously due to collateral respiration.<sup>2</sup>

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<sup>1</sup> Horaley, J. S., *J. Am. Med. Assn.*, 1925, **85**, 1209.

<sup>2</sup> Van Allen, C. M., Lindsag, G. E., and Reister, H. G., *J. Clin. Invest.*, 1937, **10**, 559.

## 7114 P

## Changes in Tissues After Intravenous Injection of Colloidal Thorium Dioxide.

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Tissue changes following intravenous injections of thorium preparations have been well described by Kadrnka and Rossier,<sup>1</sup> Irwin,<sup>2</sup> Ravenna,<sup>3</sup> Shute and Davis,<sup>4</sup> and others, but a study of very early reactions seems to have been neglected. In our studies on the effect of colloidal thorium dioxide\* upon blood and tissue cells of the rabbit, we have found that the early phase of reactions after a single intravenous injection is of great importance.

We injected 7 rabbits with 1 cc. per kg. and another 7 with a single dosage of 3 cc. per kg. They were killed 10, 15, 30 minutes, 1, 1½, 3, and 6 hours after the injection. Fresh blood smears were examined and various organs were removed and fixed in Zenker-formalin mixture. Histological sections were stained with acid fuchsin and methyl green. The green dye brings out the thorium in such a selective way that its identification inside as well as outside of the cell is greatly facilitated. In comparing the changes in the tissue of rabbits injected with 1 cc. with those of 3 cc., we found that the difference is only a matter of degree. In fact, rabbits treated with a large dosage proved to be more favorable for our purposes than cases injected with a small dosage. The following account is based upon observations in the 3 cc. dosage specimens.

Ten minutes after injection, thorium dioxide is readily identified in the spleen as a homogeneous mass of various sizes, distributed everywhere in the sinuses. Such a mass is not found in any other organ. In the specimen of 15 minutes after injection, it is found that thorium is present predominantly in the venous capillaries of the bone marrow and the liver. Here, the thorium appears to be in a free, finely granular state. The spleen at this time is full of thorium granules in the red pulp. As the latter structure is filled with slowly filtering blood, it is to be expected that more thorium particles are

<sup>1</sup> Kadrnka, S., and Rossier, J., *Acta Radiol.*, 1931, **12**, 369.

<sup>2</sup> Irwin, D. A., *Canad. Med. Assn. J.*, 1932, **27**, 130, 353.

<sup>3</sup> Ravenna, P., *Klin. Wchenschr.*, 1932, **2**, 2151.

<sup>4</sup> Shute, E., and Davis, M. E., *Arch. Path.*, 1933, **15**, 27.

\* The thorium used in this work is a commercial preparation known as "thorotrast," obtained from Chemische Fabrik von Heyden, Radebeul-Dresden, Germany.

accumulated in the spleen than elsewhere. Due to this manner of accumulation the spleen shows a definite shadow in the roentgen-film. In the 30-minute specimen the spleen contains even more thorium granules than previously, while in the liver, bone marrow, suprarenal glands, lymph nodes, etc., no appreciable change in the amount of the thorium is found. This picture remains unchanged until 1 to 1½ hour after the injection. Then, marked changes take place not only in the blood cells but also in the histiocytic elements. A sudden increase in number of polymorphonuclears is noted in the sinusoidal spaces of the spleen, in the venous capillaries of liver and bone marrow, and also in the general circulation. This condition persists for several days after the injection. Meanwhile numerous mononuclear cells contain thorium granules in the splenic sinuses. Such cells are present both in fresh smear preparations and in fixed sections. Some of them contain only a few granules while others are heavily loaded. Morphologically they resemble in many respects the large lymphocytes, except that they have more cytoplasm and many vacuoles. In the specimen of 3 hours after injection, we find no marked changes of the amount of thorium in the spleen, liver, bone marrow, and other organs. The thorium-masses are now practically in the form of granules. These are found in quite a few Kupffer cells in the liver. When the 6-hour specimen is examined, the thorium-loaded Kupffer cells become increased in number, but the presence of thorium in their cytoplasm does not distort their stellate shape. In spleen, bone marrow and lymph node the thorium granules are found to adhere to the reticular fibers as well as to the cell processes of the reticulo-endothelial meshwork. In the endothelial lining of the common blood vessels we have not found any thorium granule.

From 6 to 24 hours after the injection we have not found any change in the organs mentioned above, except that more reticular and Kupffer cells take up the thorium granules. After 24 hours, again there is no marked alteration in the amount of thorium in the different organs, but the distribution of thorium granules in the cells is of some interest. Some of the Kupffer cells and reticular cells are so heavily loaded with the thorium granules that they become round. Besides them, the hepatic cells everywhere contain very fine thorium granules. It should be pointed out that the mononuclear elements containing thorium granules in the spleen now become decreased in number.

As to the changes for periods longer than 24 hours, we have made observations on rabbits injected with 1 cc. and 3 cc. per kg.

body weight respectively, and killed them 48 hours, 1 week, 1 month, and 5 months after the injection. Our findings do not materially differ from those already reported. It is therefore unnecessary to give an account. However, two points have been insufficiently emphasized by previous reports. (1) A week or so after the injection the thorium-loaded cells always tend to group together in the form of cell-masses. Only a few of them seem to get into the lumen of the venous sinuses and are carried with the blood into the right heart and into the capillaries of the lungs, which they may obstruct. In the lung they also may be eliminated. But most of them persist in the spleen, liver, and bone marrow for a considerable time. In our histological preparations we are unable to find any elimination of such aggregated masses through the kidney. (2) A shifting of the thorium from the spleen to the liver takes place about one to 2 months after injection. In both small and large dosage specimens the thorium present in the spleen becomes decreased about 40 days after injection and meanwhile the amount in the liver is increased.

## 7115 C

### Experimental Bronchomoniliasis in Sensitized Rabbits.

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We reported<sup>1</sup> on the production of experimental bronchomoniliasis in rabbits following intratracheal administration of living cultures of pathogenic *Monilia tropicalis*. It was shown that the development of the disease was dependent upon pre-existing damage brought about by repeated intravenous injections of small doses of chaulmoogra oil. We also succeeded in producing regular sensitization of guinea pigs with cultures of various *Moniliae*. This led us to assume that perhaps sensitization of rabbits with monilia cultures may also result in an increased susceptibility of these animals to their subsequent inoculation.

The present experiment is an attempt to test this assumption. Five normal rabbits were anesthetized with ether and 0.5 cc. of a thick suspension containing 1/5 of a 2 or 15 day agar culture of *Monilia tropicalis* was injected into the trachea of the animals.

<sup>1</sup> Lim, C. E., and Kurotchkin, T. J., *Nat. Med. J. China*, 1930, **16**, 537.

Seven days later a similar injection of the same *Monilia* was repeated. For the next 2 weeks the animals showed no change in their general state. At the end of this time all animals were killed and their lungs examined; the autopsy findings indicated that those inoculated either with 2 or 15 day culture showed no detectable lesions in their lungs. Cultures made from the lungs and various parts of the large bronchi showed no growth of the *Monilia*.

A second group of 5 normal rabbits received intratracheal injection of 1 cc. of anti-monilia serum followed by 2 injections of a living culture of the *Monilia* at 7-day intervals. Two weeks later the animals were killed and examined. The result of this experiment was essentially negative, except for one rabbit injected with 15-day culture. This animal showed at the upper part of the right lung a single small, firm nodule containing a small amount of caseous white material from which a few colonies of *Monilia tropicalis* were cultivated.

A third group of 8 normal rabbits received intravenous injections of heat-killed culture of *Monilia tropicalis*. Three injections at 5-day intervals were given, each dose being equal to 1 cc. of agar culture suspended in 10 cc. of saline. Precipitin tests on the blood of all these animals performed 10 days after the last injection were positive, giving 4 plus readings with the specific carbohydrate derived from the same fungus in concentrations of 1:400,000 to 1:500,000. Considering this high precipitin content the animals were regarded as sufficiently hypersensitive. As in the previous 2 experiments, 2 subsequent inoculations of the *Monilia* were given at 7-day intervals. Three animals received 0.5 cc. each of a suspension containing 1/5 of a 2-day agar culture, and 5 animals were given the same dose of a 15-day agar culture. Two weeks after the last inoculation all rabbits were killed and the lungs examined. Two out of the 3 animals inoculated with 2-day culture presented marked nodular lesions in the upper parts of the right lung. The nodules were small, and firm and contained whitish caseous material. Microscopical examination of the stained smears prepared from the material revealed no definite *Monilia* elements, but culturally a few colonies of *Monilia tropicalis* were grown in each case. Four rabbits out of the 5, inoculated with 15-day monilia culture, developed in their lungs a nodular process, situated as in previous instances, in the upper parts of the right lung. In 2 cases the formation of nodules was associated with large and clean cavities. One rabbit developed an extensive pneumonic process involving the whole right lung. Several small nodules with caseous material were present in

the peritoneal area. In general the pathological process found in the lungs of this group of animals differed from that observed in the second group of rabbits in that the nodules were considerably larger and contained more abundant caseous material. Microscopical examination of the material from nodules was negative for *Monilia*, but culturally *Monilia tropicalis* was obtained in 2 instances.

These results suggest the conclusion that previous sensitization renders rabbits susceptible to the subsequent intratracheal administration of *Monilia tropicalis*. The lung process which may be designated as experimental bronchomycosis is confined to the formation of multiple firm nodules containing caseous material. The positive culture examination of this material indicates that the development of lung lesions is dependent upon the pathogenic activity of the fungus. A similar experiment with passively sensitized rabbits gives an essentially negative result. It is also shown that old fungous culture rich in filamentous growth is somewhat more effective in the production of lung lesions than a young culture containing mostly budding cells.

## 7116 C

### Stimulating Effect of Alum and T.A.B. Vaccine in Tetanus Prophylaxis.

W. K. CHEN. (Introduced by C. E. Lim.)

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Ramon and Zoller<sup>1</sup> in their study of active immunization against tetanus with tetanus toxoid have referred to the use of an immunizing agent consisting of toxoid plus T.A.B. vaccine on human beings in the first dose to be followed next by toxoid alone. Glenney<sup>2</sup> by the addition of potash alum to tetanus toxoid produced on experimental animals far better results than the use of the original toxoid and he attributed the increased antigenic response of alum-toxoid to delayed absorption. As alum is very irritating to the tissues, it may cause much discomfort when applied to human beings; while T.A.B. vaccine associated with toxoid will have the advantage of serving the dual function of preventive against enteric fever

<sup>1</sup> Ramon, G., and Zoller, C., *C. R. Soc. de Biol.*, 1929, **100**, 92.

<sup>2</sup> Glenney, A. T., *Brit. Med. J.*, 1920, **2**, 244.

and also a stimulus to the antigenic value of tetanus toxoid, should its stimulating effect be established as equal to that of alum. The present experiment was carried out with the object of comparing this property of alum and T.A.B. vaccine in tetanus immunization on guinea pigs.

Three groups of guinea pigs weighing about 300-350 gm. were immunized subcutaneously by 3 injections of 0.5 cc. doses of 3 kinds of antigen mixture at 14 day intervals. The first group was immunized with tetanus toxoid and T.A.B. vaccine mixture of equal parts. The second group immunized with tetanus alum-toxoid mixture (0.25% alum forming the most precipitation by titration) and the third immunized with alum toxoid (0.25% alum) and T.A.B. vaccine mixture of equal parts were utilized for comparison of their antigenic efficiency. One month after the last injection all the immunized animals (weight increased to 450-550 gm.) were tested against fresh tetanus toxin as well as the whole culture without filtration.

All the materials used in the experiment were supplied from the National Epidemic Prevention Bureau, Peiping. The tetanus toxoid was prepared by addition of 4 cc. formalin per liter of fresh tetanus toxin containing 5,000 M.L.D. per cc. and after one month incubation at 37°C. tested on guinea pigs for toxicity. The tetanus toxin was prepared by inoculating a 48-hours growth of *B. tetani* in glucose agar to 1% glucose broth (pH 7.0) under vaseline and incubating for 10 days before filtration through Berkefeld "V" candles. The triple typhoid vaccine (T.A.B.) was a saline suspension of heat-killed bacteria of 500 million *B. typhosus* and 250 million each of *B. paratyphosus* "A" and "B" per cc. with 0.25% tricresol added as preservative. The L<sub>+</sub> dose of the toxin and the M.L.D. of the whole culture were determined preliminarily on white mice of about 20 gm. in weight and also on guinea pigs of 350 gm. in weight with dosage of toxin 10 times the mouse dose. In the determination of L<sub>+</sub> dose 1, 5 of an international unit of standard tetanus antitoxin was mixed with different amounts of toxin before injection into the animals.

From the results summarized in Table I, it is evident that the antigenic efficiency of the tetanus toxoid and T.A.B. vaccine mixture is equal to that of the alum-toxoid which has been proved by many workers as the best antigen for active immunization. Also further addition of alum to the mixture of toxoid and T.A.B. vaccine does not increase the antigenic value of the original simple combination of toxoid and T.A.B. vaccine. All the animals immunized with the

## 336 ALUM AND T.A.B. VACCINE IN TETANUS PROPHYLAXIS

TABLE I.  
Active Immunization Against Tetanus.

| Antigen for immunization               | No. of animals | Test dose            | Results      |
|----------------------------------------|----------------|----------------------|--------------|
| Tetanus toxoid and T.A.B. vaccine      | 5              | 100 L+ dose toxin    | All survived |
|                                        | "              | 200 " " "            | " "          |
|                                        | "              | 400 " " "            | 1 "          |
|                                        | "              | 10 cc. whole culture | 4 died       |
|                                        |                |                      | 4 survived   |
|                                        |                |                      | 1 died       |
| Tetanus alum-toxoid                    | 4              | 100 L+ dose toxin    | All survived |
|                                        | 5              | 200 " " "            | " "          |
|                                        | "              | 400 " " "            | 2 "          |
|                                        | 4              | 10 cc. whole culture | 3 died       |
|                                        |                |                      | 3 survived   |
|                                        |                |                      | 1 died       |
| Tetanus alum-toxoid and T.A.B. vaccine | 4              | 100 L+ dose toxin    | All survived |
|                                        | "              | 200 " " "            | " "          |
|                                        | 5              | 400 " " "            | 1 "          |
|                                        | 4              | 10 cc. whole culture | 4 died       |
|                                        |                |                      | All survived |
| Control                                | 2              | 1/100 L+ dose toxin  | Died 36 hr.  |
|                                        | "              | 1/200 " " "          | " 3 days     |
|                                        | "              | 0.0002 cc. culture   | " 48 hr.     |
|                                        | "              | 0.0001 " "           | " 4 days     |

above three kinds of antigen mixtures possess a very high protective power against tetanus toxin over 200 L+ doses (or over 40,000 M.L.D.) and most of them can tolerate even a 10 cc. subcutaneous injection of a 10-day whole tetanus culture (toxin with spores).

*Conclusions.* The stimulating effect of T.A.B. vaccine in tetanus toxoid is established in the present experiment in comparison with alum-toxoid which has hitherto been recommended as the best preparation for active immunization but is unsuitable for human beings on account of its irritating action. The associated T.A.B. vaccine in tetanus toxoid is found to have the advantage of serving the dual function of a preventive against enteric fever and also a stimulus to the antigenic property of tetanus toxoid. In actual application to human cases, especially soldiers during war time, active prophylaxis against tetanus is preferable as it gives far better protection than immunization with specific antiserum. The combination of tetanus toxoid and T.A.B. vaccine for the double prophylaxis against tetanus and enteric fever by a series of 3 injections is, therefore, to be recommended to military hygienists for further trial.

## 7117 P

## Antihistamine Activity of Whole Blood.

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Although the rôle of histamine in anaphylaxis is still disputed, the resemblance between symptoms in fatal anaphylaxis and histamine shock has been pointed out by Dale and Laidlaw<sup>1</sup> and histamine-like substance in anaphylaxis has been demonstrated in guinea pig by Bartosch<sup>2</sup> and in dog by Gebauer-Fuelnegg and Dragstedt.<sup>3</sup> In the present study an attempt has been made to compare the histamine destroying power (histaminase<sup>4</sup>) of whole blood of guinea pig, rabbit, dog and man, and to see if any correlation may be made between the humoral destruction of histamine of these species and their relative susceptibility to histamine.

Freshly drawn blood samples were defibrinated by shaking with glass beads for 5 to 10 minutes. Varying amounts of blood were mixed with 1 cc. normal saline containing 1 mg. histamine acid phosphate (ergamine) and saline was added to make volume of the samples uniform in each experiment. After adding a few drops of toluene, the mixture was incubated at 37°C., for 24 hours. At the end of incubation the histamine content in the mixture was determined by Dale's blood pressure method (Burn<sup>5</sup>) in atropinized and doubly vagotomized cats under luminal anesthesia. Control blood samples (without addition of histamine solution), blood samples heated to 70°C. for 10 minutes before addition of histamine solution, and standard histamine solution (without addition of blood) were also incubated for 24 hours at 37°C. before the test.

No control blood samples (without addition of histamine) showed any depressor effect on the cat, and no heated blood samples any histamine destruction activity. From the blood of 22 guinea pigs, 10 rabbits, 3 dogs, and 3 men, the following results were obtained. The rabbit blood showed maximum histamine destruction at 5 cc., dog at 10 cc. On the other hand, guinea pig blood showed no de-

<sup>1</sup> Dale, H. H., and Laidlaw, P. P., *J. Physiol.*, 1919, **52**, 355.

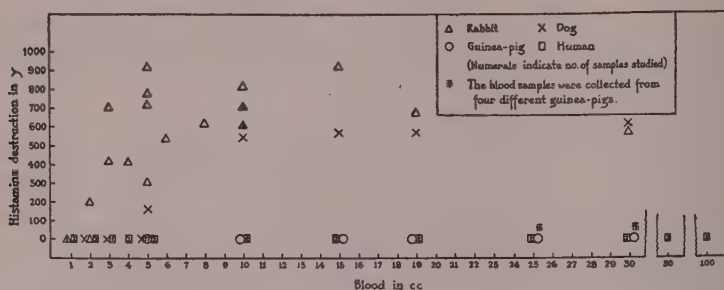
<sup>2</sup> Bartosch, R., Feldberg, W., and Nagel, E., *Arch. f. d. ges. physiol.*, 1932, **230**, 129, 674.

<sup>3</sup> Gebauer-Fuelnegg, E., and Dragstedt, *Am. J. Physiol.*, 1932, **102**, 520.

<sup>4</sup> Best, C. H., and McHenry, E. W., *J. Physiol.*, 1930, **70**, 349.

<sup>5</sup> Burn, J. H., *Methods of Biol. Assay*, 1928, IX, 111.

tectable destruction of histamine at 30 cc. Similarly, human blood showed no destruction of histamine up to 100 cc. Graph 1 gives the actual data.



In view of the fact that the guinea pig is more susceptible to fatal histamine shock than either the dog or rabbit (Schmidt and Stähe-<sup>6</sup>lin<sup>6</sup>), it may be suggested that the sensitivity to histamine in guinea pig and man may be accounted for by the absence of antihistamine activity in their blood. One may possibly attribute the high susceptibility of the guinea pig to anaphylaxis (assuming that the toxic symptoms of anaphylaxis are due to a histamine-like substance) to the absence of anti-histamine activity in its blood. Since the susceptibility of man to anaphylaxis can not be subjected to experimental test, the present finding on human blood may perhaps be taken as evidence of the susceptibility of man to anaphylaxis.

<sup>6</sup> Schmidt, G. W., and Stähelein, A., *Z. f. Immunitätsforsch.*, 1929, **60**, 222.

## New York Meeting.

*New York Academy of Medicine, December 20, 1933.*

7118 C

### Experimental Evidence of an Additional Substance Essential to Mammalian Nutrition.

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*From the Department of Chemistry, Columbia University.*

It has become increasingly evident that one or more water soluble substances other than vitamins B ( $B_1$ ) and G ( $B_2$ ) were necessary for general mammalian nutrition. Further evidence appeared during the course of an experiment to study the effect of fairly high concentrations of vitamin G upon growth and reproduction. Litter mates had been divided between 3 diets planned to be both adequate and practically equal in all the known nutritional essentials except for the one variable, vitamin G. From previous experience it was expected that early growth would be improved by the higher concentrations of vitamin G.

It soon became apparent, however, that vitamin G had ceased to be the limiting factor since inferior growth attended the higher concentrations of that vitamin. At 140 days of age the average weight of the 4 males was 318, 269, and 250 gm., and that of the 6 females 239, 209, and 182 gm. for the 3 diets which contained successively increasing concentrations of vitamin G. This reversal was carried over to the young whose average weight at 28 days of age was 57 (35 cases), 47 (22 cases), and 29 (7 cases) gm. for the males, and 53 (31 cases), 43 (20 cases), and 30 (8 cases) gm. for the females, notwithstanding successively increasing amounts of vitamin G. The average gain in weight of representative young of the second generation from the 28th to 56th day of life was 119 (6 cases), and 89 (3 cases) gm. for the males, and 80 (6 cases), and 68 (6 cases) gm. for the females. The diet richest in vitamin G was not represented here.

When representative young from the 3 diets were fed Bourquin

and Sherman's<sup>1</sup> vitamin G deficient diet at 28 days of age, a striking reversal of their growth occurred. Those young which had grown poorly and were from mothers showing poor growth now grew the best. The average gain in weight from the 28th to 56th day was 9 (9 cases), 10 (10 cases), and 16 (9 cases) gm. for successive increases of vitamin G in the previous dietary.

It appeared, therefore, that growth had been previously inhibited on the diets richer in vitamin G by the deficiency of a substance present in the Bourquin and Sherman vitamin G deficient diet. The alcoholic extract of whole wheat seemed the most probable carrier of this nutritionally essential substance.

From a study of its properties and distribution, this substance may be identical with or closely allied to Reader's<sup>2</sup> vitamin B<sub>4</sub> and to the factor reported by Halliday.<sup>3</sup> It is present in whole wheat but only in lesser degree in skimmed milk powder since the poorest growth was obtained on the diet containing 70% skimmed milk powder by weight. It may be, therefore, the same factor which Halliday found in whole wheat but probably different from the factor which Coward<sup>4, 5</sup> found to be potent in milk. Its presence in the whole wheat extract demonstrates its extractability by ethyl alcohol, 80% by weight. Reader reported that her vitamin B<sub>4</sub> was most readily extracted with 50% alcohol but to some extent by ether. Likewise, this substance must be relatively stable to heating at 78°C. for 2 hours in alcoholic solution. Reader has reported that the activity of her crystalline vitamin B<sub>4</sub> was destroyed by heating with alkali but not with acid, and that her crude vitamin B<sub>4</sub> was destroyed by 2 hours open heating at 95-97°C. to the extent of about 50%.

The concentration of this additional factor must have been fairly low in the diet attended by very inferior growth, but during the 6 months of the experiment no external symptoms other than lowered vitality appeared.

<sup>1</sup> Bourquin, A., and Sherman, H. C., *J. Am. Chem. Soc.*, 1931, **53**, 3501.

<sup>2</sup> Reader, V., *Biochem. J.*, 1929, **23**, 689; 1930, **24**, 77, 1827; Barnes, H., O'Brien, J. B. P., and Reader, V., *Biochem. J.*, 1932, **26**, 2035.

<sup>3</sup> Halliday, N., *J. Biol. Chem.*, 1932, **96**, 479.

<sup>4</sup> Coward, K. H., Key, K. M., and Morgan, B. G., *Biochem. J.*, 1929, **23**, 695.

<sup>5</sup> Coward, K. H., Key, K. M., Morgan, B. G., and Cambden, M., *Biochem. J.*, 1929, **23**, 913.

## 7119 C

# Reaction of Transplantable and Spontaneous Tumors to Blood-carried Bacterial Toxins in Animals Unsusceptible to the Shwartzman Phenomenon.

F. DURAN-REYNALS. (Introduced by J. B. Murphy.)

*From the Laboratories of the Rockefeller Institute for Medical Research.*

Gratia and Linz<sup>1</sup> described a phenomenon characterized by a violent hemorrhagic and necrotic reaction in the tumor in guinea pigs bearing large transplantable lipi-sarcomas, shortly after intravenous injection of a filtrate of *B. coli* broth culture. The authors identified this phenomenon with that of "Sanarelli-Shwartzman", and also with similar local hemorrhagic reactions resulting from intravenous injection of bacterial filtrates in rabbits injected by a number of bacteria and filterable viruses.\* Shwartzman and Michalowsky<sup>2</sup> described the same phenomenon in a rather extensive series of mice bearing Sarcoma 180. Guinea pigs as well as mice may die within 24 hours following the injection.<sup>3-5</sup> Some of the mouse tumors may temporarily regress or completely disappear.

Besides their intrinsic value, 2 considerations make these findings particularly interesting: (1) mice and rats are unsuceptible to the ordinary Shwartzman phenomenon, and it would seem that special conditions existing in the tumor render its vessels, supplied by the host, apt to react with the blood-carried bacterial toxins. (2) This state of reactivity of the tumor in either susceptible or non-susceptible animals is a permanent one, without the need of any previous local injection of bacterial filtrate as is the case with the ordinary Shwartzman phenomenon in rabbits and guinea pigs.

We have studied the phenomenon described by Gratia and Linz on a variety of neoplastic processes in animals unsuceptible to the Shwartzman phenomenon, namely, rats and mice. As a source of bacterial toxin we used filtrates of 6-day-old broth cultures of *S. coli* and injected them into the peritoneum or vein in the amount of 0.5 cc. for mice and 1 cc. for rats. We have divided our results into 2

<sup>1</sup> Gratia, A., and Linz, R., *Compt. rend. Soc. Biol.*, 1937, 108, 427; *Ann. Inst. Pasteur*, 1932, 49, 131.

\* As early as 1907 S. P. Beebe and M. Traup<sup>3</sup> described the regression of several cases of dog lymphosarcoma under the influence of the "Coley toxin" from *Streptococcus B. coli*, and *B. prodigiosus*.

<sup>2</sup> Beebe, S. P., and Traup, M., *J. Am. Med. Assn.*, 1907, 49, 1493.

<sup>3</sup> Shwartzman, G., and Michalowsky, N., *Proc. Soc. Exp. Biol. and Med.*, 1932, 29, 737.

groups, according to whether the phenomenon was positive or negative.

*1st group (positively reacting tumors).* This group includes 203 mice or rats bearing rapidly growing malignant transplantable tumors which give a high percentage of takes and rarely retrogress. The tumors differed widely in age, size, and degree of necrosis at the time of the toxin injection. The results are summarized in Table I.

TABLE I.

| No. of animals | Tumor                | % showing positive phenomenon | % of regressed tumors | % death within 24 hours following injection |
|----------------|----------------------|-------------------------------|-----------------------|---------------------------------------------|
| 61 mice        | S/37 sarcoma         | 67                            | 20                    | 0                                           |
| 68 "           | 180 "                | 60                            | 10                    | 10                                          |
| 21 "           | M/63 adeno-carcinoma | 57                            | 0                     | 47                                          |
| 19 "           | Twort " "            | 70                            | 0                     | 0                                           |
| 34 rats        | Walker sarcoma       | 88                            | 0                     | 41                                          |

In the tumors included in Table I the intensity of the phenomenon bears a direct relationship with the age and size of the tumors. While very young, perfectly healthy tumors often give a negative phenomenon, large tumors, averaging 1x1 cm. for mice and 2x1 cm. for rats, give practically 100% positive results. The strongly positive phenomenon is characterized by an extensive hemorrhagic condition, which in most cases may be observed externally through the skin. A more moderate phenomenon requires the opening of the growth to be detected. The very necrotic parts, where vessels are obliterated, are naturally free from hemorrhage. In the more prominent cases the phenomenon can be detected in the tumor as early as one hour after the peritoneal injection of bacterial filtrate.

*2nd group (negatively reacting tumors).* This group includes 69 mice and rats bearing: (1) comparatively slowly growing spontaneous or transplantable malignant tumors which rarely or never regress, (2) rapidly growing malignant tumors which eventually regress (heterologous graft), and (3) benign embryomas or granulomas, rapidly developing, which eventually regress. The detailed account is as follows:

19 mice bearing spontaneous malignant mammary adeno-carcinomas.

6 mice bearing the transplantable malignant Harding and Passey melanotic sarcoma.

4 mice bearing the transplantable malignant Walker rat tumor.

10 rats bearing the transplantable malignant S/37 or 180 mouse sarcoma.

20 mice bearing benign embryomas.

10 mice bearing kieselguhr granulomas.

None of these tumors or granulomas showed any definite hemorrhagic phenomenon. Only 2 spontaneous tumors and 2 mouse tumors growing in rats showed a doubtful mild phenomenon. Three spontaneous tumors were tested a second time 24 hours after the tumor had been "prepared" by injection of 0.2 cc. of filtrate into the mass. Again no modification in the tumor could be noticed. It is worth pointing out that some of the mouse tumors growing in rats had at the time of injection attained a mass of  $2.2 \times 0.9$  cm. of perfectly healthy tissue with actively dividing cells and apparently perfect blood supply, as shown by histological examination. Such growths were the result of grafting large pieces of tumors under the skin of the heterologous host.

From the results obtained with the tumors so far studied, it appears that only those growths showing at the same time *malignancy* and *rapidity of growth* show the phenomenon of Gratia and Linz.

The mechanism of death as a result of the reaction in the tumor is not understood, and no significant general lesions have been found at the autopsies. Numerous control tests have shown that normal mice and rats stand the injection of the bacterial filtrate without marked alterations in their health. Additional experiments have shown that the general resistance of the animals to the ordinary Shwartzman phenomenon is not changed by the existence of the tumor. Also small tumors may be found unsusceptible despite their growing in an animal bearing large susceptible tumors. The frequency of death seems to be in a direct relationship with the size and age of the tumor, but besides this there is another intrinsic factor depending on the strain of tumor itself. The Bashford adenocarcinoma seems, from this point of view, to be the most "toxic" of all the strains so far studied.

Animals in which, as a consequence of the phenomenon, the tumors regressed were found resistant to regrafting. The intimate cause of this tumor regression is now being investigated.

In view of the fact that transplantable tumors are often infected by various bacteria, we have secured sterile tumors, and found that they are as sensitive to the phenomenon as the infected ones. Moreover, filtrates of tumors showing a strong phenomenon, injected into the skin of rabbits, have failed to sensitize this organ to intravenous injection of a potent bacterial filtrate made 24 hours later.

Work is underway extending these studies to more true tumors and granulomas occurring in animals both susceptible and resistant

to the ordinary Schwartzman phenomenon in order to obtain more information about the relationship existing between malignancy and rapidity of growth on the one hand, and ability to react to bacterial toxins on the other hand.

*Summary.* Whereas rapidly growing transplantable malignant tumors in rats and mice are very susceptible to blood-carried *B. coli* toxin, slow-growing malignant spontaneous or transplantable tumors, malignant tumors rapidly growing in heterologous hosts, embryomas, and granulomas are practically non-susceptible.

## 7120

### Contribution to the Etiology of Encephalitis. Differentiation of Encephalitis by Protection Tests.

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*From the Laboratories of the Rockefeller Institute for Medical Research.*

Muckenfuss, Armstrong, and McCordock<sup>1, 2</sup> and Webster and Fite<sup>3</sup> have reported that the encephalitis epidemic in St. Louis during the summer and autumn of 1933 is communicable, by inoculation, to monkeys and mice. In addition, Webster and Fite<sup>3</sup> reported that the encephalitis prevailing in Kansas City at the same time is likewise communicable to mice; that the infectious agent from the St. Louis and Kansas City cases is filterable, is readily transmissible to mice, is highly virulent when instilled into the nasal passages of mice, and is neutralized by the serum of encephalitis convalescents from the 1933 epidemic.

We have continued our studies of the effect on the encephalitis virus of various sera derived from cases of encephalitis and from immunized monkeys, and will report the result of these tests in the present paper.

Monkeys injected with the virus develop in their sera protective properties similar to those in the sera of convalescent St. Louis and Kansas City encephalitis cases. Again, serum from a monkey injected with one strain of virus from a St. Louis case protects not only against that strain of virus but against 2 other strains from St.

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<sup>1</sup> Leake, J. P., *J. Am. Med. Assn.*, 1933, **101**, 928.

<sup>2</sup> Muckenfuss, R. S., Armstrong, C., and McCordock, H. A., *Pub. Health Rep.*, 1933, **48**, 1341.

<sup>3</sup> Webster, Leslie T., and Fite, George L., *Science*, 1933, **78**, 463.

Louis and 1 from Kansas City. Finally, the monkey serum prepared with our St. Louis strain of virus protects against 2 strains of the Muckenfuss, Armstrong, McCordock virus.\*

Sera from one monkey immunized with poliomyelitis virus and one monkey convalescent from experimental poliomyelitis† did not protect against the encephalitis virus. Serum from a patient with herpes likewise showed no protective properties.‡

Sera from 34 cases in the encephalitis ward in the St. Louis City Isolation Hospital, August to October, 1933, have been tested.§ Of these, 30 were definitely diagnosed encephalitis and 4 questionable encephalitis complicated by schizophrenia, hysteria, hypertension, and malaria, respectively. These 4 later cases were negative when tested. Of the 30 with a definite diagnosis of encephalitis, 3 from whom sera were obtained within 7 days of onset of symptoms were negative; 5 of 12 from whom sera were drawn 7 to 14 days after onset were positive, 41%; and 14 of 15 from whom sera were drawn 7 to 41 days after the recorded onset of symptoms were definitely protective, 93%. Sera from 4 of 5 tested convalescent cases of encephalitis in Kansas City showed protective properties.\*\*

Sera from 2 convalescent cases of meningoencephalopathy in Indianapolis, studied by Kempf, Gilman, and Zerfas,<sup>4</sup> did not protect. Sera from 13 cases in New York, New Jersey, and Connecticut, diagnosed acute encephalitis in September, 1933, showed no protective properties.†† Sera from 16 cases, diagnosed chronic epidemic encephalitis with Parkinsonian sequelae, tested 1 to 13 years after onset of symptoms, did not protect.‡‡

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\* Two strains of the Muckenfuss, Armstrong, McCordock virus in mouse brains were sent to us by Dr. Muckenfuss for comparative tests. Both strains set up a disease in our mice similar to that produced by our virus.

† We are indebted to Dr. Simon Flexner for these sera.

‡ This serum, as well as sera from chronic cases diagnosed epidemic encephalitis, was obtained from the Medical Service of the New York Hospital through the courtesy of Dr. Du Bois.

§ We are indebted to Dr. Muckenfuss for the brain tissue and to Drs. J. W. Eschenbrenner, Jr., and S. Weisman for sera from cases of encephalitis in St. Louis.

\*\* We are indebted to Dr. Paul Stookey for sending sera from convalescent cases and brain tissue from fatal cases of encephalitis prevailing in Kansas City in 1933.

<sup>4</sup> Kempf, G. F., Gilman, L. H., and Zerfas, L. G., *Arch. Neur. and Psych.*, 1933, **29**, 433.

†† We thank Drs. Frissell and Green of the St. Luke's Hospital, Dr. Josephine Neal and the Matheson Commission, Dr. Marine and the Neurological Service of the Montefiore Hospital, Dr. Raynor and Bloomingdale Hospital, Dr. Von Oehsen of Bradley Beach, N. J., and Drs. F. Blake and J. Fox of the New Haven Hospital for these sera.

On the other hand, sera from 5 tested cases of encephalitis in an outbreak in Paris, Illinois, occurring in the summer of 1932 and described by Conklin,<sup>5</sup> did show protective properties. And finally, serum from 1 case of acute encephalitis, September, 1933, in Dr. L. F. Frissell's service at St. Luke's Hospital, New York, thought by him to resemble clinically the St. Louis type, and from 1 case of acute encephalitis, September, 1933, seen by Dr. H. T. Chickering in New York, showed protective properties against the virus.

The results indicate, first, that the filterable virus obtained from fatal cases of encephalitis in St. Louis and Kansas City is the specific agent responsible for the encephalitis epidemics in St. Louis and Kansas City during the summer of 1933 and the epidemic in Paris, Illinois, during the previous summer; second, that the cases of meningoencephalopathy in Indianapolis were probably different from the encephalitis in St. Louis; third, that cases of the St. Louis type of encephalitis occurred in New York during September, 1933; and finally, that chronic cases of so-called epidemic encephalitis thus far tested do not show protective properties in their sera against this encephalitis virus.

## 7121 P

### On the Motility of the Colon of the Dog.

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A new technique was devised, for the purpose of studying separately the movements of the 2 coats of the intestine of the unanesthetized dog. The results obtained necessitate a revised statement of the "Law of the Intestine", at least for the colon of the dog.

A section of the colon about 10 cm. long was exteriorized; opened along its anti-mesenteric border, and grafted onto the subcutaneous tissue of the anterior abdominal wall. The mucosal surface now presents outward, so that stimuli may be applied to it in imitation of intra-colonic conditions. The motility of the coats of the graft is recorded by modified enterograph levers. The principle involved is that of recording the change in distance between two clips attached to the mucosa, either longitudinally or transversely to the graft.

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<sup>5</sup> Conklin, W. E., personal communication.

The stimulus which was used in the present study was a stroke applied to the mucosa by means of a blunt metal teaser. The experiments were performed on non-anesthetized animals, after they had recovered from the operation, and had been trained to lie quietly on their sides.

Longitudinal irritation of the mucosa of the colon graft elicited contraction of the longitudinal muscle, with little or no response from the circular muscle. Irritation of the mucosa transversely elicits a response from both coats of the colon. The longitudinal muscle contracts *first*, and is *followed* by contraction of the circular muscle. The biological significance of this order of contraction is made evident by the following observations.

When the transverse stroke stimulus is applied to the mucosa cephalad to the recording clips, the longitudinal muscle contracts at and *below* the area of stimulation; there is no contraction above the stimulus. The circular muscle contracts at and *above* the area of stimulation; there is no contraction below. The longitudinal contraction *below* the stimulus precedes the circular contraction *above* the stimulus by 3 to 5 seconds. The "myenteric reflex" is abolished by atropine given systemically, and by cocaine applied to the mucosa of the graft at the area of stimulation.

Contraction of the longitudinal muscle, acting alone, enlarges the lumen of the gut. When this occurs *below* the point of stimulation, the downward passage of the bolus is facilitated.

Contraction of the circular muscle, acting alone, narrows the lumen of the gut. When this occurs *above* the stimulus, the passage of the bolus orad is prevented. Occurring after the gut lumen is enlarged below the bolus, it promotes the downward passage of the bolus. As the bolus passes downward, a new area is stimulated, and new reflexes are initiated. Such a sequence of reflex responses may give the appearance of peristalsis.

The "law of the intestine" or "myenteric reflex" may be restated as follows: In the colon of the unanesthetized dog, *stimuli applied to the mucosa elicit first a contraction of the longitudinal muscle at and below the point of stimulation; this is followed by contraction of the circular muscle at and above the site of stimulation.*

## Metabolism of Various Sulfur Compounds in Cystinuria.\*†

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A cystinuric patient was kept for a period of approximately 4 months on a standard meat free diet and his urine analyzed daily for various nitrogenous and sulfur constituents. In addition the cystine sulfur was determined by both the Folin and the Sullivan<sup>1</sup> methods.

During experimental periods of 3 days each the following compounds were fed in the quantities indicated containing equivalent amounts of sulfur: cystine (6.4 gm.), glutathione (16 gm.), cysteine HCl (8.8 gm., neutralized) and dl-methionine (8 gm.). The experiments were carried out in triplicate except the one with glutathione which was repeated only once.

The results may be summarized as follows:

*Cystine* experiments showed that 94% of the extra sulfur was excreted as inorganic sulfate and that there was no rise in the excretion of cystine.

*Glutathione* experiments showed that 80% of the extra sulfur was excreted as inorganic sulfate and that there was a slight rise in the excretion of cystine which amounted to 9% of the extra sulfur.

*Cysteine* experiments showed that only 34% of the extra sulfur were excreted as inorganic sulfate while there was a large increase in the excretion of cystine, continuing for several days after the feeding, which increase in cystine excretion accounted for 66% of the extra sulfur. The analyses also indicated that additional cystine and not cysteine was being excreted.

*dl-Methionine* experiments showed that only 34% of the extra sulfur was excreted as inorganic sulfate while there was a large increase in the excretion of cystine, continuing for several days after the feeding, which increase in cystine excretion amounted to 47% of the extra sulfur; there was also a definite increase in the excretion

\* Aided by Grants Nos. 181 and 302 from the Committee on Scientific Research of the American Medical Association.

† Presented before the Division of Biological Chemistry at the Fall Meeting of the American Chemical Society, Chicago, September 14, 1933.

<sup>1</sup> Cf. Brand, E., Harris, M. M., and Biloon, S., *J. Biol. Chem.*, 1930, **86**, 315.

of undetermined (non-cystine) neutral sulfur which amounted to 19% of the extra sulfur. The analyses again indicated that additional cystine and not cysteine was being excreted.

These experiments tend to show that the metabolism of cystine may be quite different from that of cysteine and that these 2 compounds are not as interchangeable in intermediary metabolism as has been assumed generally. They further show that the metabolic behavior of an amino acid may vary markedly depending upon whether it is catabolized as a free amino acid (cysteine) or in combined form as a peptide (glutathione). The experiments also indicate that methionine may be metabolized via cysteine. The experiments thus indicate the possibility of a conversion of methionine into cystine. The experiments by Jackson and Block<sup>2</sup> and by White and Lewis,<sup>3</sup> which have established a metabolic interrelationship between methionine and cystine perhaps should be interpreted in this way.

The finding that cystine is excreted following the administration of cysteine indicates the possible rôle of the kidney in the oxidation reduction mechanism of the SS-SH system.

It appears that the cystine which the cystinuric excretes in the urine may be derived, in part or in whole, from that portion of the protein sulfur which is present in the protein molecule in the form of methionine. Further experiments will be necessary to determine to what an extent cystinuria is primarily a disturbance in methionine metabolism.

The possibility of a disturbance in glutathione synthesis in cystinuria should be considered. This view would be in keeping with the aspects of intermediary protein metabolism indicated in a previous publication.<sup>4</sup>

## 7123 P

### Cultivation of European Type of Typhus Rickettsia in Presence of Live Tissue.

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The cultivation of Rickettsia presents considerable difficulties. Successful cultivation, with demonstrable rickettsia in cells, has been

<sup>2</sup> Jackson, R. W., and Block, R. J., *J. Biol. Chem.*, 1932, **98**, 465.

<sup>3</sup> White, A., and Lewis, H. B., *J. Biol. Chem.*, 1932, **98**, 607.

<sup>4</sup> Brand, E., and Harris, M. M., *Science*, 1933, **77**, 589.

reported in tissue cultures by Wolbach and Schlessinger,<sup>1</sup> and Zinsser and Batchelder.<sup>2</sup> Pinkerton and Hass,<sup>3</sup> and Nigg and Landsteiner<sup>4</sup> were the first to obtain positive subcultures through several successive generations, the latter in flask or Maitland cultures. The last named authors worked with strains producing scrotal lesions in guinea pigs and used the tunica vaginalis infiltrated with rickettsia as the infective material. Pinkerton and Hass, though presumably working with a European strain, report the production (though irregular) of scrotal lesions in guinea pigs.

Our attempts to cultivate a European strain\* by the methods used by Pinkerton and Hass or Nigg and Landsteiner failed to give positive cultures. Brain as well as tunica tissue was used as infective material but no rickettsia grew out. We, therefore, changed the procedure and used infected lice intestines for starting the culture.

In the experiments reported below successful initial cultures as well as subcultures were obtained by using infected lice intestines as infective material and following in all other respects the procedure employed by Nigg and Landsteiner. Apparently the positive outcome of these experiments is due to the use of heavily infected lice tissue, containing large numbers of rickettsia, to initiate the culture.

The technique was briefly as follows: Infected lice, 8-10 days after having been infected by the Weigle technique, were sterilized by treatment with alcohol and the guts removed aseptically into a drop of saline in a sterile hollow slide. Normal guinea pig tunica was macerated in the drop together with the infected louse gut and the mixture allowed to stand for 15-30 minutes. Small amounts of this material were then placed in small rubber-stoppered flasks containing 3 cc. tyrode-serum (2.5 cc. tyrode solution and 0.5 cc. fresh guinea pig serum). At first the cultures were placed at 37°C., but subsequently they were kept at 30°C. At the latter temperature cultures as well as transplants were obtained. The following typical protocols give the procedure and the results obtained thus far:

*Experiment 1.* Normal guinea pig tunica was macerated with 3 intestines of heavily infected lice and allowed to stand for half an hour. The minced tissue was distributed in small flasks containing

<sup>1</sup> Wolbach, S. B., and Schlesinger, M. J., *J. Med. Res.*, 1923, **44**, 231.

<sup>2</sup> Zinsser, H., and Batchelder, A. P., *J. Exp. Med.*, 1930, **51**, 847.

<sup>3</sup> Pinkerton, H., and Hass, G. M., *J. Exp. Med.*, 1931, **54**, 307.

<sup>4</sup> Nigg, C., and Landsteiner, K., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 3.

\* Obtained from Dr. Breinl's laboratory. This is a typical European strain giving characteristic brain but no scrotal lesions. Scraping of the tunica failed to reveal rickettsia.

2.5 cc. Tyrode solution and 0.5 cc. fresh guinea pig serum and placed at 37° C. After 8 days a heavily infected endothelial cell was found in one of the flasks. The culture failed, however, to infect a guinea pig.

*Experiment 2.* Cultures were set up as above on 16/6 and 19/6 respectively and incubated at 30° C. After 8 and 9 days, respectively, fairly large numbers of rickettsia were found within cells as well as free near ruptured endothelial cells. After 13 days two of the positive cultures were mixed, the tissue ground, mixed with fresh minced tunica from a normal guinea pig and allowed to stand 30 minutes. The subcultures were set up as above and incubated at 30° C. Fourteen days later rickettsia were found in endothelial cells in the subculture. A guinea pig was infected with material from the positive cultures and after an incubation period of 12 days it developed a typical infection. A passage was made to another guinea pig which came down after 8 days and lice infected with the brain emulsion of this animal showed after 7 days numerous rickettsia in the epithelial cells of the intestine.

A successful transplant was, therefore, made from the first culture and the subculture was viable and infective 27 days after the initial culture was made. It should be noted that a suspension of rickettsia—infected louse intestines in serum tyrode solution without guinea pig tunica loses its infectivity for guinea pigs in 2 days at 30° C. Moreover, since the endothelial cells of the tunica in the cultures were found heavily packed with rickettsia it is evident that growth must have taken place. It seems reasonably certain that the European type of Typhus rickettsia can be grown in flask cultures containing guinea pig tunica and incubated at 30°C.

## 7124 P

### Spectral Absorption of Purified Tuberculin.

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*From the Department of Colloid Chemistry, D. J. McCarthy Foundation, Temple University School of Medicine and the Henry Phipps Institute, University of Pennsylvania.*

The characteristic spectral properties of normal proteins and their derivatives stimulate similar studies with material showing special

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\* Aided by a grant from the National Tuberculosis Association

biological activity. Previous investigations along a similar line were hampered by the use of impure material.<sup>1</sup> Therefore, for the studies here presented several preparations of tuberculin, purified according to the methods of Seibert and Munday,<sup>2</sup> were used.

Preparations made from human, bovine and avian tubercle bacilli were studied. In the case of the human tubercle bacillus there were 5 different fractions: (1) 3 purified unheated tuberculins, (2) a purified Old Tuberculin, and (3) a purified Old Tuberculin made from cultures in a synthetic medium. Products made similarly from the non-pathogenic acid-fast bacilli, timothy, butyricum and smegma, and purified protein, proteoses, and a cyclic amino acid, served as controls. The initial concentration of all of these slightly alkalinized solutions was one per cent. In these studies a small Quartz Hilger spectrograph was used. Carbons containing iron provided the spectrum.

In all preparations made from the 3 strains of tubercle bacilli, the spectrograms showed a specific absorption band with a maximum at 265 m $\mu$ . The concentration at which this band appeared varied in the different preparations and seemed to correspond closely to their respective tuberculin potencies. The more potent preparations showed the special absorption band at a higher dilution than the less active samples. This was observed not only in the case of the 3 different tubercle bacillus strains but also in different fractions from the same strain (human). The preparations made from the 3 non-pathogenic strains of bacilli failed to show this special absorption band.

Irradiation with ultra-violet light, known to destroy the potency of tuberculin, seems to increase the total absorption and at the same time destroys the specific absorption band.

The detailed data and spectrograms with a discussion concerning their significance and suggestions for further practical use will be presented elsewhere.

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<sup>1</sup> Fischer, A., and Hausmann, W., *Z. Tuberk.*, 1930, **58**, 328.

<sup>2</sup> Seibert, F. B., and Munday, B., *Am. Rev. Tub.*, 1932, **25**, 724.

## 7125 C

## Effect of Reticulo-Endothelial Blockade on Blood Chemistry.

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*From the Department of Pathology, University of Illinois, College of Medicine.*

The rôle of the reticulo-endothelial system in immunity, antibody formation and anaphylaxis has been the subject of many investigations. Apart from its place in blood pigment and cholesterol metabolism, however, there have been relatively few and somewhat contradictory reports on the metabolic function of this group of cells. A hypocholesterolemia was observed by Leites<sup>1</sup> and by Goebel and Gnoinski.<sup>2</sup> Gnoinski<sup>3</sup> found a hyperglycemia, Demont<sup>4</sup> observed a hypoglycemia. Sterken and Kerner-Pashenyan<sup>5</sup> saw no change in the blood sugar level following colloidal injections. DuBois<sup>6</sup> observed a drop in albumin and total nitrogen but no constant change in the non-protein nitrogen and we<sup>7</sup> have previously shown a rise in the blood uric acid level following reticulo-endothelial blockade.

This diversity depends upon a variety of factors, the species of animal used, the amount and character of material injected, and the length of time allowed to elapse following injections. The most important factor, however, in the interpretation of results is whether the reticulo-endothelial cells are stimulated or depressed by the injections. Whether they can be completely blocked is still open to question. Jaffe<sup>8</sup> believes that a temporary functional depression is possible, and is most marked during intracellular segregation of the electro-negative colloid. Small amounts of colloidal material are supposed to stimulate and large amounts to depress but it is not clearly known what may be considered a small or a large amount.

We have conducted many experiments on rabbits and dogs using India ink and sacharrated iron oxide as blocking agents and believe the former to give better results. Here we report experiments with India ink into dogs.

An 8% suspension of India ink and normal saline was continu-

<sup>1</sup> Leites, *Biochem. Z.*, 1927, **186**, 391.

<sup>2</sup> Goebel and Gnoinski, *Compt. Rend. Soc. de Biol.*, 1927, **96**, 1483.

<sup>3</sup> Gnoinski, *Compt. Rend. Soc. de Biol.*, 1928, **98**, 72.

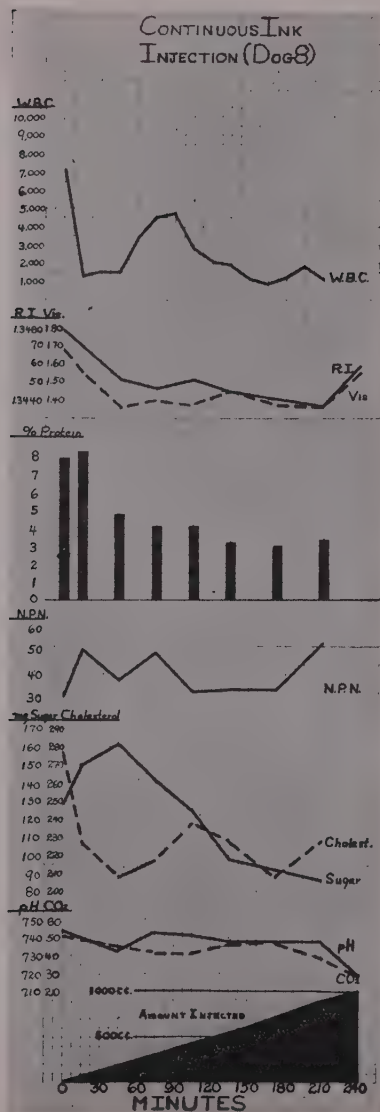
<sup>4</sup> Demont, *Compt. Rend. Soc. de Biol.*, 1926, **95**, 916.

<sup>5</sup> Sterken and Kerner-Pashenyan, *Zhur. Exptl. Biol. Med.*, 1929, **10**, 431.

<sup>6</sup> DuBois, *Compt. Rend. Soc. de Biol.*, 1931, **108**, 637.

<sup>7</sup> Klein and Levinson, *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 885.

<sup>8</sup> Jaffe, *Physiol. Rev.*, 1931, **11**, 277.



ously injected into the femoral vein of a dog. White blood counts were made and blood drawn before the injection and at various intervals during the injection. The total non-protein nitrogen, cholesterol and sugar, the plasma pH,  $\text{CO}_2$ , and total proteins and

the refractive index and viscosity of the serum were determined. All of the animals showed similar results as Dog 8.

The immediate response to injection is a sharp drop in the white blood count and cholesterol and a rise in the sugar. The pH, CO<sub>2</sub>, and refractive index and viscosity fall more slowly. There is a noticeable peripheral vaso-constriction and increased splanchnic activity due, no doubt, to the reticulo-endothelial stimulation. In 45 minutes, however, we see a definite attempt at re-establishment of equilibrium. The white blood count tends to return to normal, the drop in refractive index, viscosity, protein and cholesterol is arrested. The pH returns to its previous level. The blood sugar which had increased now begins to fall. This is due, in all probability, to the exhaustion of the available supply of glycogen. This period, we believe, is one of attempted re-establishment of equilibrium as the organism brings all of its histiocytic elements into play.

The continuous injection, however, overcomes the attempts of the organism to cope with the constant stimulation and fatigue and functional paralysis with ultimate death follows. This is reflected in the secondary fall in the white blood count, final rise in non-protein nitrogen and the terminal acidosis. These results are strikingly similar to those obtained by Petersen and his associates<sup>9</sup> during the continuous intravenous injection into dogs of a suspension of *B. coli* and leads us to believe that such changes can be due to the effect on the reticulo-endothelial system alone. It must not be forgotten, however, that India ink contains a protein which we have been able to detect by the biuret test and which may play a rôle.

*Summary.* The continuous intravenous injection of India ink into dogs over a certain period of time results in a series of changes in the blood and tissues characterized by 3 stages of reaction: (1) an initial period of reticulo-endothelial stimulation and increased splanchnic activity. (2) an attempted re-establishment of equilibrium. (3) reticulo-endothelial exhaustion, the premortal phase.

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<sup>9</sup> Petersen, Mueller and Boikan, *J. Inf. Dis.*, 1927, **41**, 405.

## Response in Sinistrally Ovariectomized Leghorns to Daily Injections of Hebin.\*

L. V. DOMM. (Introduced by Frank R. Lillie.)

*From the Whitman Laboratory of Experimental Zoology, University of Chicago.*

We reported the precocious development of sexual characters in the juvenile Leghorn female following homeoplastic hypophyseal implants<sup>1</sup> as well as injections of hebin.<sup>2</sup> As a result of the daily injection of hebin, head furnishings responded by a phenomenal growth becoming large and masculine in character similar to that of treated males. Plumage and spurs exhibited no modifications, neither were changes observed in behavior. Postmortem revealed prominent ovaries which were larger and heavier than controls though revealed no signs of ovulation. Oviducts revealed pronounced hypertrophy comparable to that preceding ovulation. Thyroids were likewise conspicuously hypertrophied.

In an attempt to extend these observations 2 problems interested us particularly. The first was the source of the hormone causing masculinization of head furnishings in treated females. It was assumed that the medullary tissues of the rudimentary right gonad, as well as those of the left ovary, hypertrophied and elaborated male hormone. That this is a plausible explanation was revealed by our results following sinistral and dextral ovariectomy<sup>3</sup> in which the hypertrophied medullary tissues of either side were found to exert a masculinizing influence on head furnishings. Our results following sinistral ovariectomy logically would lead one to suppose that the masculinization of head furnishings following injections of hebin in the female is the result of hypertrophy of the rudimentary right gonad. However, since this organ exhibited relatively very little hypertrophy following these injections it was not considered as the major source of the hormone causing growth of head furnishings in these experiments. The second problem concerned a possible solution for the sporadic occurrence of spermatogenesis in

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\* This investigation was supported in part by a grant from the committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

<sup>1</sup> Domm, L. V., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 310.

<sup>2</sup> Domm, L. V., and van Dyke, H. B., *Proc. Soc. Exp. Biol. and Med.*, 1932, **30**, 351.

<sup>3</sup> Domm, L. V., *J. Exp. Zool.*, 1927, **48**, 31.

the hypertrophied right gonad of early sinistrally ovariectomized Leghorns. Why does spermatogenesis occur in such a small percentage of cases in such individuals? Is it due to an insufficiency of gonad-stimulating hormone, during an early period when the primordial germ cells are still present, preventing active growth of the rudimentary right gonad and consequent incorporation of these cells? If so, then it might be expected that daily administration of hebin, immediately following early sinistral ovariectomy, would produce a greater percentage of fertile right testes in such individuals. Experiments were devised which might facilitate a solution of these problems.

Four small groups of light brown Leghorn females were ovariectomized and 2 to 3 days later we began giving them daily subcutaneous injections of hebin.† Ages on the day injections began were 21, 28, 35, and 105 days, and duration of injections 21, 36, 38, and 42 days. Concentrations administered were 10 and 20 rat units in single daily injections. Birds were weighed and head furnishings measured at regular intervals.

Unlike our results following similar injections in the normal female the poultard revealed absolutely no response of head furnishings in these experiments. In a few of the older, longer treated poultards there were indications of a slight response near the end of the experiment but these were not regarded as significant since a similar response appeared at about the same time in a few of the controls. The assumption of male plumage did not appear to be inhibited. No perceptible modifications were revealed by either spurs or behavior.

Postmortem, as in the normal, showed some hypertrophy of the rudimentary right gonad though it is questionable whether this greatly exceeds that of controls. However, when compared with the conspicuous hypertrophy of the left ovary in treated normals the hypertrophy here seemed rather insignificant. The oviduct was apparently not modified, for its weight fluctuated little above or below that of controls. Thyroids, however, exhibited significant hypertrophy, which in some cases was quite pronounced.

These observations make it evident that the rudimentary right gonad supplies very little, if any, of the hormone responsible for the masculinization of head furnishings in the normal treated females of our experiments and show that its major source must be the left ovary. Further investigations are being made on these problems in which greater concentrations are being employed.

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† Purchased through Dr. David Kline from The Wilson Laboratories, Chicago.

### Response in Unilateral and Bilateral Castrate Leghorns to Daily Injections of Hebin.\*

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We previously reported the precocious development of sexual characters in cockerels following homeoplastic hypophyseal implants<sup>1</sup> and later a similar effect was reported following daily injections of hebin.<sup>2</sup> These experiments not only revealed a profound response on the part of head furnishings and behavior but primary and accessory sexual characters were affected as well. Following injections of hebin head furnishings soon became many times larger than controls and masculine in character. Cockerels crowed as early as 9 days of age and began treading at 13. On postmortem testes and ductus deferens revealed definite and frequently pronounced hypertrophy. Thyroids likewise were larger and heavier than controls. Histological examination of testes revealed significant modifications. The tubules of treated testes were, in all cases examined, distinctly larger and more advanced in development and in many cases revealed complete spermatogenesis.

In view of these interesting results on normal cockerels it was considered advisable to study the effects of similar experiments in unilateral and complete castrates. Accordingly a group of left unilateral castrates, a group of right unilateral castrates, and some capons were given daily subcutaneous injections of hebin. Ages at the time injections began were 28 and 35 days. Castrations in all cases having been done 2 to 3 days before the injections began. The duration of injections was 21 and 22 days. The dosages were 10 and 20 rat units in single daily injections. All individuals in the experiments were weighed and head furnishings measured at regular intervals. Treated birds were not adversely affected by the injections.

It was found that unilateral castrates responded much as did normals following daily injections of hebin. Pronounced growth of head furnishings was manifested just as early as in normals and was

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\* This investigation was supported in part by a grant from the committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

<sup>1</sup> Domm, L. V., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 308.

<sup>2</sup> Domm, L. V., and van Dyke, H. B., *Proc. Soc. Exp. Biol. and Med.*, 1932, **30**, 349.

equally pronounced. The capon, however, revealed no such response. The head furnishings here remained small and pale throughout and revealed no growth. Behavior was likewise unmodified, remaining typically capon throughout.

Postmortem revealed hypertrophy of the remaining testis in both unilateral castrate groups. The testes here were larger and heavier than those of comparable controls. The following comparative weights of testes from treated and control unilateral castrates are of interest. The average weight of a group of 5 left testes from treated birds was 1.044 gm., while that of 6 controls was 0.106 gm., of 6 treated right testes 0.658 gm., and that of 6 controls 0.121 gm. The heaviest treated left testis weighed 2.339 gm. to 0.240 gm. for the control; the heaviest treated right 1.398 gm. to 0.326 gm. for control. The ductus deferens revealed hypertrophy in treated cases. Thyroids likewise exhibited hypertrophy in both groups of treated unilateral castrates. The average thyroid weight of the left testis group above is 0.075 gm. for treated to 0.014 gm. for controls and for the right testis group above 0.105 gm. for treated to 0.017 gm. for controls.

The only organ exhibiting unquestionable response in the capon was the thyroid. This organ, as in normals, was usually considerably larger and heavier in treated capons. Capon No. 235, 28 days old when injections began, received 10 rat units daily for 21 days and was killed on the day following the last injection. Its thyroids at this time weighed 0.215 gm. while those of 2 controls weighed 0.028 gm. and 0.034 gm. respectively.

Our observations thus indicate that unilateral castration does not inhibit the response of sexual characters, observed in normals following injections of hebin,<sup>†</sup> while complete castration does. Castration, however, did not inhibit a thyroid response. Our results thus indicate a direct action on gonads and thyroids and show that the hypertrophy of gonads in turn is responsible for the development of sexual characters.

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<sup>†</sup> Purchased through Dr. David Kline from The Wilson Laboratories, Chicago.

# Amount of Material Effective in Pernicious Anemia Present in Dog Liver.

MAURICE B. STRAUSS AND WILLIAM B. CASTLE. (Introduced by Robert N. Nye.)

*From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Departments of Medicine and Tropical Medicine, Harvard Medical School.*

Addisonian pernicious anemia may be considered to be a dietary deficiency disease usually conditioned by the absence of a specific "intrinsic factor" from the gastric secretion.<sup>1</sup> It is believed that in the normal person or animal the interaction of this gastric factor which is thermolabile, and a dietary factor associated in many substances with the vitamin B complex, results in the storage of a thermostable substance in the liver. The presence of the thermolabile factor has been demonstrated in human<sup>1, 2</sup> and bovine<sup>3</sup> gastric juice and in the stomach tissues of the hog.<sup>4</sup> Thermostable material has been found in the livers of these 3 species,<sup>5, 6</sup> as well as in those of sheep,<sup>6</sup> deer,<sup>6</sup> horses,<sup>7</sup> and fish.<sup>8</sup> Although present in greatest concentration in the liver, the thermostable material is also found in kidney,<sup>6</sup> brain,<sup>9</sup> placenta<sup>10</sup> and other organs. The demonstration of the presence of either the thermolabile or the thermostable material depends upon the typical response of suitable patients with pernicious anemia to the administration of the organs or their extracts referred to above. In the case of the thermolabile factor an opportunity for contact with the dietary factor under appropriate conditions is essential to the test.<sup>1</sup>

<sup>1</sup> Castle, W. B., and Townsend, W. C., *Am. J. Med. Sc.*, 1929, **178**, 764.

<sup>2</sup> Wilkinson, J. F., *Brit. Med. J.*, 1930, **1**, 236. Barnett, C. W., *Am. J. Med. Sc.*, 1931, **182**, 170. Singer, K., *Klin. Wchnschr.*, 1932, **11**, 1459.

<sup>3</sup> Williams, H. A., and Vanderveer, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 858.

<sup>4</sup> Sharp, E. A., *J. Am. Med. Assn.*, 1929, **93**, 749. Sturgis, C. C., and Isaacs, R., *J. Am. Med. Assn.*, 1929, **93**, 747.

<sup>5</sup> Unpublished Observations.

<sup>6</sup> Minot, G. R., Murphy, W. P., and Stetson, R. P., *Am. J. Med. Sc.*, 1928, **175**, 578.

<sup>7</sup> Richter, O., Meyer, A. E., and Ivy, A. C., *J. Am. Med. Assn.*, 1932, **98**, 1623.

<sup>8</sup> Connery, J. E., *Am. J. Med. Sc.*, 1930, **180**, 603. Davidson, L. S. P., *Brit. Med. J.*, 1932, **2**, 347.

<sup>9</sup> Ungley, C. C., *Lancet*, 1931, **2**, 63.

<sup>10</sup> Mach, R. S., *Sang*, 1931, **5**, 299.

Gutzeit and Herrmann<sup>11</sup> have reported finding the specific intrinsic factor in the gastric juice of the dog. On the contrary, Ivy and his associates<sup>12</sup> have failed to obtain any effect in Addisonian pernicious anemia by the administration of desiccated dog stomach and have also failed to produce pernicious anemia in the dog by total gastrectomy.<sup>13</sup> If the dog's gastric juice contains the specific intrinsic factor, it is reasonable to assume that "liver extract", potent in Addisonian pernicious anemia, ought to be stored in the livers of normal dogs. If, on the contrary, the dog's gastric juice does not contain the intrinsic factor, as is suggested by the observations of Ivy, the dog's liver should not contain the thermostable principle. In order to determine the "liver extract" content of canine liver, 4 identical extracts suitable for intramuscular injection were prepared from normal dog livers. For control purposes 4 similar extracts were prepared from normal hog livers. The method of preparation of each of these extracts was as follows: The original quantity of liver pulp, as well as the volumes of filtrates and concentrates at each stage of the process, was kept as nearly as possible the same in each preparation. Fresh liver was finely ground, and the pulp separated from the fibrous tissue in a separatory grinder. Canine extract No. 1 was made from a single dog liver. Portions of the livers of 10 different dogs were used in the preparation of canine extract No. 2, and of 4 and 3 different dogs in the preparation of canine extracts No. 3 and No. 4, respectively. The pulp was added to twice its weight of tap water, the mixture stirred vigorously and allowed to stand over night in a refrigerator. The mixture, after being heated rapidly to the boiling point, was acidified to pH 6, and filtered through hard paper by suction, following which the residue was re-extracted with half the original quantity of water and again filtered. Sufficient 95% alcohol was added to the combined filtrates to make a final concentration of 70% alcohol. The filtrate, after standing over night, was then separated from the precipitate and evaporated *in vacuo* at a temperature of less than 45° C. to a small volume. Sufficient 95% alcohol was then added to make the concentration 70%, the filtrate was again separated from the precipitate and sufficient absolute alcohol added to make the final concentration 95% alcohol. The precipitate, after sedimenting over night, was washed with ether, dried and dissolved in water so that 10 cc. of the solution was derived from 100 gm. of fresh liver pulp. The

<sup>11</sup> Gutzeit, K., and Herrmann, J., *München. med. Wchnschr.*, 1931, **78**, 266.

<sup>12</sup> Ivy, A. C., Richter, O., and Kim, M. S., *Proc. Am. Physiol. Soc., Am. J. Physiol.*, 1932, **101**, 59.

<sup>13</sup> Ivy, A. C., Morgan, J. E., and Farrell, J. I., *Surg. Gynec. Obst.*, 1931, **53**, 611.

TABLE I.

Daily Administration by Intramuscular Injection of Liver Extracts Derived from the Amounts of Fresh Liver Indicated.

| Days | No. 1, 20 gm. |            | First Periods. Canine Extract. |            | No. 3, 100 gm. |            | No. 4, 100 gm. |             |
|------|---------------|------------|--------------------------------|------------|----------------|------------|----------------|-------------|
|      | Case No. 1    | Case No. 2 | Case No. 2                     | Case No. 3 | Case No. 3     | Case No. 4 | Case No. 4     | Case No. 4  |
|      | R.B.C.        | Retics.    | R.B.C.                         | Retics.    | R.B.C.         | Retics.    | R.B.C.         | Retics.     |
|      | mils.         | %          | mils.                          | %          | mils.          | %          | mils.          | %           |
| 0    | 1.86          | 1.8        | 2.05                           | 0.6        | 1.68           | 1.8        | 1.51           | 0.6         |
| 2    | 1.78          | 2.2        | 2.08                           | 0.8        | 1.52           | 2.2        | 1.37           | 0.6         |
| 4    | 1.73          | 0.6        | 2.11                           | 0.8        | 1.85           | 5.0        | 1.51           | 1.2         |
| 6    | 1.74          | 0.1        | 2.18                           | 2.0        | 1.92           | <b>8.8</b> | 1.55           | 7.2         |
| 8    | 1.46          | 1.0        | 2.20                           | 2.2        | 2.00           | 6.2        | 1.51           | 17.8        |
| 10   | 1.39          | 1.2        | 2.17                           | <b>4.4</b> | 2.33           | 4.6        | 1.74           | <b>20.6</b> |

|    |               | Second Periods. Hog Extract. |                |                |      |     |      |
|----|---------------|------------------------------|----------------|----------------|------|-----|------|
|    | No. 1, 20 gm. | No. 2, 25 gm.                | No. 3, 100 gm. | No. 4, 100 gm. |      |     |      |
| 2  | 1.38          | 2.2                          | 2.40           | 3.6            | 2.31 | 1.2 | 1.92 |
| 4  | 1.44          | 2.8                          | 2.32           | 2.0            | 2.27 | 0.8 | 1.96 |
| 6  | 1.51          | 6.6                          | 2.34           | 1.8            | 2.41 | 1.8 | 2.24 |
| 8  | 1.92          | 10.7                         | 2.23           | 1.4            | 2.85 | 1.0 | 2.38 |
| 10 | 1.98          | <b>13.5</b>                  | 2.58           | 1.0            | 2.80 | 1.2 | 2.36 |
| 12 | 2.05          | 7.8                          | 2.68           | 1.2            | 2.72 | 0.8 | 2.75 |

reaction was adjusted to pH 7.4, 0.3% tricresol added and the material passed through a Berkefeld filter, following which it was stored in sterile vials kept in a refrigerator.

The results of the intramuscular administration of these extracts are shown in Table I. To each of 4 patients canine liver extract was administered for 10 days and then immediately followed during the next 10 days by hog liver extract. No demonstrable effect, either upon the reticulocytes or upon the red blood corpuscles, was obtained when the extract from 20 gm. of canine liver was injected daily in Case 1. Subsequently, in the same patient, a satisfactory response occurred following the administration of a similar extract derived from the same quantity of hog liver. The second patient, Case 2, showed a small reticulocyte response and a moderate gain of erythrocytes following the daily injection of extract derived from 100 gm. of dog liver. During the second period, this patient was given hog liver extract daily in amounts derived from 25 gm. of liver. No secondary reticulocyte response occurred. The third patient, Case 3, showed a moderate response of reticulocytes and a gain of over half a million red blood cells in a period of 10 days, during which daily injections of the extract derived from 100 gm. of dog liver were administered. A similar amount of hog liver extract was administered to this patient during the subsequent period with no second reticulocyte response. The fourth patient, Case 4, showed a maximum reticulocyte response during the daily

intramuscular injection of the extract derived from 100 gm. of dog liver, without a secondary increase of reticulocytes during the administration of similar amounts of hog liver extract.

These observations demonstrate that the liver of the dog contains thermostable material effective in pernicious anemia, although in lesser quantities than does hog liver. As far as we have been able to ascertain, practically all other mammalian livers thus far assayed, have contained the same relative concentration of effective material. The hog, ox, sheep, horse and deer, however, are all essentially herbivorous animals, whereas the dog belongs to the carnivora. A partial deficiency of either the dietary or the gastric factors could theoretically account for the diminished quantity of potent substance in the liver of the dog. Judging from their state of nutrition, the diet of these animals appeared to be satisfactory and, therefore, presumably contained adequate amounts of the factor associated with the vitamin B complex. On the other hand, the contradictory observations<sup>11, 12</sup> cited above, upon the content of intrinsic factor of the dog's stomach, suggest an explanation for the decreased potency of the dog liver. It is possible that a decreased amount of intrinsic factor is present in the gastric juice of the dog and that this results in a decreased amount of thermostable effective material in the liver.

*Conclusions.* 1. Extracts made from normal dog livers produce on intramuscular injection typical remissions in patients with Addisonian pernicious anemia. 2. The content of potent material in canine liver appears to be only about one-fifth that of hog liver. 3. This fact may possibly be correlated with the contradictory findings concerning the amount of the specific intrinsic factor in canine gastric juice.

7129 P

### Treatment of Pellagra by Means of Parenteral Liver Extract.

TOM D. SPIES. (Introduced by J. T. Wearn.)

*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, and the Medical Service, Lakeside Hospital, Cleveland.*

Goldberger and Sebrell<sup>1</sup> found that liver extract fed to dogs in large amounts either prevented or retarded the development of

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<sup>1</sup> Goldberger, J., and Sebrell, W. H., *J. Am. Med. Assn.*, 1932, **99**, 95.

blacktongue (a disease which they consider analogous to pellagra of human beings). Boggs and Padgett<sup>2</sup> stressed the curative value of liver (not liver extract) in pellagra and Smith and Ruffin<sup>3</sup> stated that large amounts of liver extract by mouth were efficacious in treating the disease. Ramsdell and Magness<sup>4</sup> in a preliminary study on the effects of intramuscular liver extract on the course of pellagrins, gave small daily doses and at the same time allowed the patients a highly nutritious diet. They felt that the intramuscular administration of liver extract resulted in clinical improvement but stated the patients were not given a basic diet. Since many pellagrins have severe oral lesions and refuse to eat, it seemed worthwhile to determine the therapeutic efficacy of intravenous liver extract under controlled experimental conditions.

Six patients with the classical skin and oral lesions of pellagra were selected for the first part of the experiment. Each patient was placed on a pellagra-producing diet until the stomatitis and glossitis became worse. Then 80 cc. of liver extract\* were administered intravenously in 4 divided doses in the subsequent 20 hours without any change in the basic diet. Four other patients were controlled in an identical manner and intramuscular liver extract\* was injected in 3 divided doses of 10 cc. each.

The tongue and oral mucous membranes, which had become worse on the basic diet, appeared less red and swollen within 24 hours after the first parenteral treatment of liver extract. Seventy-two hours after the injection, either intravenous or intramuscular, the lesions were healed.

It has been shown in this experiment that the stomatitis and glossitis of 6 pellagrins responded to intravenous liver extract and that the oral lesions of 4 other pellagrins were likewise healed by intramuscular liver extract. It is suggested that liver extract be used as a parenteral therapeutic agent whenever a severe pellagrin has difficulty in ingesting or assimilating adequate amounts of a highly nutritious diet.

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<sup>2</sup> Boggs, T. R., and Padgett, P., *Bull. Johns Hopkins Hosp.*, 1932, **50**, 21.

<sup>3</sup> Smith, D. T., and Ruffin, J. M., *J. Clin. Invest.*, 1933, **12**, 963.

<sup>4</sup> Ramsdell, R. L., and Magness, W. H., *Am. J. Med. Sciences*, 1933, **185**, 568.

\* Furnished through the courtesy of Dr. E. A. Sharp, Parke, Davis & Co.

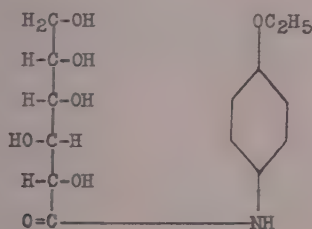
## 7130 C

# Comparison of the Antipyretic Action and Toxicity of D-Glucono-Para-Phenetidin and Acetphenetidin.

W. E. HAMBOURGER. (Introduced by H. G. Barbour.)

From the Department of Pharmacology and Toxicology, Yale University.

D-Glucono-Para-Phenetidin\* is a gluconic acid derivative of phenetidin, having the following structure:



*Glucono-phenetidin.* This is analogous to acetphenetidin, the acetic acid derivative. The structural similarity of these 2 compounds suggested a comparison of their antipyretic activities, using fevered rabbits, and of their toxicities, using white rats.

*Antipyretic Action.* The 2 drugs were tested on rabbits fasted for 48 hours and without water for 24 hours, which were fevered by the injection of hay infusion. Five experiments were conducted. The procedure of a typical experiment was as follows: 14 animals were selected and rectal temperatures taken. Two animals were set aside as normal, room temperature controls, and received no further treatment. The remaining 12 animals, weighing between 2 and 3 kg., were injected subcutaneously with 5 cc. per kg. of a 10% hay infusion, incubated for 24 hours at 37°. Two hours after injection, rectal temperatures were again taken, and the 3 animals with the poorest fevers were discarded. The 9 remaining animals, which had fever rises ranging from 0.80° to 2.30° C., were then separated into 3 groups: (a) Three animals were kept as fever controls; (b) 3 animals were treated with glucono-phenetidin; (c) 3 animals were treated with acetphenetidin. The drugs were suspended in 50 cc. of 1% acacia solution, and were fed by stomach tube. The 3 fever control animals received a similar administration of acacia solution. Rectal temperatures were taken periodically over 6 hours after the

\* The Glucono-phenetidin used in this study was kindly furnished by Charles Pfizer & Co., Inc., New York.

feeding. In the first experiment a dosage of 0.300 gm. per kg. of acetphenetidin and a corresponding molecular weight (7:4) of glucono-phenetidin (0.525 gm. per kg.) were used; these doses proved to be about minimal. In one of the remaining experiments, doses of 0.500 gm. per kg. of acetphenetidin and 0.875 gm. per kg. of glucono-phenetidin were used, and in the remaining 3 experiments, respective doses of 0.600 gm. and 1.050 gm. were used.

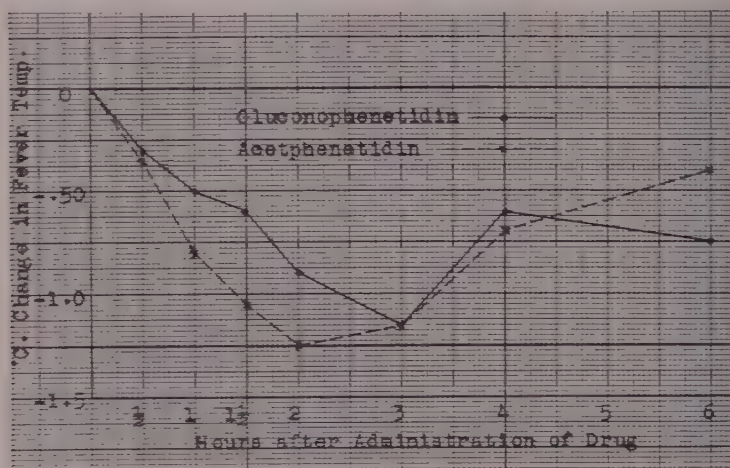


FIGURE 1.

Comparison of antipyretic activity of glucono-phenetidin and acetphenetidin on fevered rabbits. Summary of 3 experiments (see text), glucono-phenetidin 1.050 gm. per kg.; acetphenetidin 0.600 gm. per kg.

The data in each group for each experiment were found to be consistent and average values could therefore be taken, the temperature changes in the room- and fever-control animals being used to correct the temperature changes produced by the drugs. Figure 1 graphically presents such average values for the 3 experiments with the largest dose (1.050 gm. per kg. of glucono-phenetidin, and 0.600 gm. per kg. of acetphenetidin). This represents 9 animals for glucono-phenetidin, 8 for acetphenetidin, 7 fever controls, and 7 normal controls. The ordinate of the curves indicates the corrected average changes in the initial fever temperatures, which are represented by the zero mark: the horizontal line through this mark, indicating the corrected average fever control temperatures. The curves show very little difference in the antipyretic action of the 2 drugs. The one experiment, with doses of 0.875 gm. of glucono-phenetidin and 0.500 gm. per kg. of acetphenetidin, compared with

the larger doses of the preceding experiment, gave a slower but similar effect for the 2 compounds.

*Toxicity.* These studies were made on rats, after a one-day fast, fed by stomach tube with emulsions of each of the 2 drugs in 5% acacia solution. Glucono-phenetidin was used on 20 animals with doses ranging up to 50 gm. per kg. of body weight. All survived the treatment for from 4 to 6 days (the length of the period of observation) without any other signs than an occasional immediate slight depression, apparently due to the large volume which was fed (in a few cases above 5 cc.). It is conceivable that the larger bulk of the glucono feedings may have diminished its absorption mechanically, and so decreased its toxicity: but no toxicity was observed even with doses 10 times as large as those for acetphenetidin.

Definite signs of toxicity were observed with acetphenetidin. The drug was fatal only slowly (more than 4½ hours). Toxic signs frequently occurred within 2 hours with the higher doses, and more slowly with the smaller doses. Seventeen animals were fed. The minimal fatal dose was about 4 to 6 gm. per kg. by stomach tube. Post mortem examination of 2 animals disclosed only slight pulmonary edema and no distinctive lesions. Death seemed to be due essentially to some blood dyscrasia which produced cyanosis. Other symptoms were a slow deep respiration with occasional wheezing and dripping of clear fluid from the nose, diarrhea and coma, with occasional muscular twitchings of a clonic nature.

*Summary.* The antipyretic action of glucono-phenetidin in equimolecular proportions is about equal to the action of acetphenetidin when tested on rabbits fevered with injections of hay infusion. Glucono-phenetidin is non-toxic to rats in doses up to 50 gm. per kg., whereas acetphenetidin manifests occasional toxic symptoms in doses as low as 4 gm. per kg., and was fatal in 3 out of 5 animals with a dose of 6 gm. per kg. This indicates a definitely wider range between therapeutic and toxic dose for glucono-phenetidin than for acetphenetidin.

## A Note on the Mode of Action and Administration of Vitamin D.

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Albany Medical College.

Lewis and Zotterman<sup>1</sup> have suggested that ultraviolet light forms in the skin a histamine-like substance which, presumably, would increase the gastric secretions. Also, various investigators have reported a decreased alkalinity in the intestinal contents during exposure to ultraviolet light. Furthermore, it has already been demonstrated that acidity in the gastrointestinal tract, by making calcium salts more soluble, has a healing effect on rickets. Therefore, we thought that vitamin D might increase the absorption of calcium and phosphorus by stimulating the secretion of gastric juice.

Standard rachitic rats were given from 0.005 to 0.5 mg. of histamine subcutaneously, twice each day for 21 days. The smaller doses of the drug caused no noticeable change in the appearance and general behavior of the animals. On the other hand, the larger doses increased the respiration, heart rate and especially the secretions from the eyes so that these rats appeared "watery-eyed" throughout the experimental period. Roentgenograms of the different groups of rats, taken at the end of treatment, showed no evidence of healing when compared with the controls. Thus it is apparent that vitamin D does not cure rickets by acting on the gastric juice.

These experiments support the findings of Bauer *et al*<sup>2</sup> and Hottinger,<sup>3</sup> who were unable to explain the healing action of vitamin D on the basis of an increased formation of acid in the gastrointestinal tract.

We also made a comparative study of oral and subcutaneous administration of vitamin D. Although Kramer and his associates<sup>4</sup> have cured rickets by subcutaneous injection of cod liver oil in ether solution, the relative efficiency of the 2 methods of treatment

\* George Alexander Research Fellow in Biochemistry, 1930-32, sponsored by The Leopold Schepp Foundation, New York.

<sup>1</sup> Lewis, T., and Zotterman, Y., *Heart*, 1926, **13**, 203.

<sup>2</sup> Bauer, W., Maddock, S. J., and Wood, J. C., *Am. J. Med. Sci.*, 1931, **181**, 399.

<sup>3</sup> Hottinger, A., *Strahlentherapie*, 1929, **34**, 637.

<sup>4</sup> Kramer, B., Kramer, S. D., Shelling, D. H., and Shear, M. J., *J. Biol. Chem.*, 1927, **71**, 699.

was not compared. We found that both procedures were equally efficient in healing rickets when 0.1 to 0.2 mg. of viosterol were given to rachitic rats daily for 21 days. However, when the equivalent of 21 doses of vitamin D was administered in one dose on the first day, or a third of the total amount on each of the first, seventh, and tenth days, it was observed that the subcutaneous method of treatment brought about a complete cure while oral administration resulted only in moderate or advanced healing. This was true even when the viosterol for a particular day was given in 3 portions of 0.1 cc. each in order to insure a more accurate dosage.

These observations indicate either incomplete absorption of vitamin D from the intestine, when given in large amounts by mouth, or a mechanism less efficient for storage than that formed when the vitamin is administered subcutaneously in ether solution.

Wilkins and Kramer<sup>5</sup> have already shown that the injection technique is practical, and our results demonstrate further that it is possible to inject large doses of viosterol without loss of effectiveness. Therefore, it seems evident that subcutaneous administration would be advantageous in clinical treatment where difficulty is experienced with oral administration. Using a highly concentrated preparation of viosterol or cod liver oil, sufficient vitamin D could be given in 2 or 3 injections to either cure or prevent rickets.

Fifty-three rats were used in the 2 series of experiments. Rats 28 days old weighing from 40 to 60 gm. were kept on the Steenbock rachitic diet for 21 days, at the end of which time a marked degree of rickets developed. The rats were then weighed, X-ray pictures taken, and the experimental period started. After 21 days the final weight was recorded and X-ray pictures were again taken. In doubtful cases the line test was used as a check on the roentgenograms. Only those rats gaining, or at least maintaining their weight during the experimental period were considered in our studies.

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<sup>5</sup> Wilkins, L., and Kramer, B., *Bull. Johns Hopkins Hosp.*, 1927, 40, 52.

### Fertility and Development of Newt Eggs Obtained after Anterior Lobe Implants.

ESTHER M. PATCH. (Introduced by M. F. Guyer.)

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Five pairs of adult Newts of the species *Triturus viridescens*, Woods Hole strain, were transferred on January 6, 1932, from the water in which they were received at 5°C. to aquaria at 20°C. Courtship by the males followed and at least one spermatophore was deposited on the sand in each of 2 aquaria; the females, however, were not incited to follow far and the uncollected sperm cell masses were scattered by the movements of the animals. No egg-laying occurred. After a week, the males were removed from the aquaria and the females were given daily implants of anterior lobe of amphibian pituitary to stimulate ovulation (Wolf<sup>1</sup>). Anterior lobes obtained from Newts of the same species, from *T. dorsalis*, and from the frog, *Rana pipiens*, were placed intramuscularly as in the work of Adams,<sup>2</sup> one lobe being used for each implant. Only non-fertile eggs were deposited; egg-laying ceased when implants were stopped. Untreated isolated males deposited no spermatophores.

Males of *Triturus* were stimulated to deposit whole spermatophores or small clusters of sperm cells (not always mature and active) by 3 methods: (1) isolated males after 2 and after 3 daily implants of amphibian anterior lobe; (2) isolated males after 4 and after 6 daily injections intraperitoneally of gonadotropic hormone as extracted from human pregnant urine; (3) males paired in aquaria with females, when the preliminary period of courtship was shortened if either had received implants. After the sperm cell masses were gathered from the bottom of the aquaria by a small glass pipette and were transferred to the cloacas of 4 treated females (with 5, 5, 6, and 2 daily implants), they laid fertile eggs. Three of the females began to deposit eggs within a day, the fourth after an interval of 8 days during which 2 more implants were given; fertility after this delay would seem to indicate that some of the spermatozoa inserted had made their way to the spermatheca. The low degree of fertility obtained by this method (142 of 365 eggs from the 4 fe-

<sup>1</sup> Wolf, O. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 692; *Anat. Rec.*, 1929, **44**, 206.

<sup>2</sup> Adams, A. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 433; *Anat. Rec.*, 1930, **45**, 250.

males), in addition to the lack of fertility without pipette transfer of sperm, suggests that no spermatozoa had been collected during the fall mating season and that the few present to fertilize the eggs were those retained from the laboratory procedure.

Fertile eggs were usually attached to the water plants; those which dropped to the bottom of the aquaria had been forced out after too short a stay in the cloaca for fertilization to occur. There was marked individual variation not only in the number and fertility of the eggs laid (33 fertile of 48 laid, 57 of 120, 32 of 160, 20 of 37), but also in the size and degree of their maturity, in the duration of the egg-laying period (6, 17, 6, and 4 days), and in the relation of this period to the total number of implants received (9, 10, 7, and 4, respectively). The third female produced, during the last 2 days of her egg-laying, very small immature eggs, unpigmented and pressed end to end in a long trailing albuminous strand.

Isolated female Newts from the same source given implants in February, 1933, laid only non-fertile eggs. Non-treated females laid no eggs. Further work was prevented by deaths of both sexes, probably due to the 2 species of tapeworms found on autopsy.

The embryos developing from the induced eggs of January, 1932, showed a high degree of asymmetry, appearing for the most part before the closure of the neural folds. Duplications of balancers or of gills, supernumary or fused digits were frequent. Except for these latter minor variations, 94 animals were selected as apparently normal upon arrival at the eating stage and were reared for 6 weeks on ostracods, 2 species of daphnids, and enchytreids. Synthetic diets, used for the remaining period of larval growth, led to a range in length from 32 to 47 mm. at the time of metamorphosis.

### 7133 P

#### Precipitin and Fermentation Reactions of the Moniliae.

JOHN HENDERSON LAMB AND MARGARET LAIN LAMB. (Introduced by J. Howard Brown.)

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In the course of the study of smears from cases of bronchial asthma and routine cultures taken from cases of ringworm infection of the feet, numerous yeast-like organisms were encountered, especially

those of the monilia group. Since fermentation reactions and serological tests proved to form a very important basis for the identification of the moniliae, as well as other yeasts, further studies have been made of these 2 characteristics.

Previous investigators, in the study of fermentation reactions, have made their observations on extract broth plus 1% fermentable sugars with Andrade's solution as an indicator, and Durham tubes for determination of gas. Because of the differences in the results of these authors, some method seemed necessary to investigate this test further. Pork infusion fermented broth (the carbohydrates in the meat infusion were removed by fermentation with *B. coli* before the peptone was added) was used with addition of the various carbohydrates. The medium was inoculated with monilia and incubated at 37°C. The pH of the inoculated media was followed every 2 or 3 days for 20 to 40 days by the colorimetric method described by Brown.<sup>1</sup> Eight or 10 drops of the uninoculated media gave distinctly positive reactions for sugar with Benedict's solution. Those cultures found to give a negative chemical test for sugar after a period of incubation were considered to have fermented the carbohydrates.

The hydrogen-ion concentration of the cultures of moniliae in 2% sugar broth over a period of 40 days showed a wide divergence in the amount of acidity produced in the fermenting process. Several strains, although showing little if any acidity, fermented the sugar as shown by its disappearance indicated by the negative Benedict's test. Taking the disappearance of the sugars as a basis for division, the moniliae fall into 3 groups:

A first group, *M. albicans*, *M. candida* and *M. psilosis*, utilizes completely sucrose, dextrose, maltose, xylose, levulose and galactose, but does not attack inulin. A second group including *M. parapsilosis* and *M. type 1* (Stovall),<sup>2</sup> utilizes 4 of the above sugars not fermenting maltose or inulin and rarely xylose. A third group includes *M. krusei* which does not utilize xylose, sucrose, maltose, galactose or inulin.

As a further step in identifying these organisms precipitin reactions were investigated. Previous descriptions of methods used for the making of extracts of these fungi for the test were time-consuming and complicated. A simpler method was devised. The organisms were cultivated on Sabouraud's agar in Petri dishes. After 48 hours the heavy surface growth was scraped off, emulsified in 10

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<sup>1</sup> Brown, J. H., *J. Lab. and Clin. Med.*, 1924, 9, 23.

<sup>2</sup> Stovall, W. D., *J. Infect. Dis.*, 1932, 50, 73.

cc. of normal saline to which one-half cc. of N/1 HCL was added. This suspension was boiled for 15 minutes and centrifuged. The supernatant fluid was drawn off, neutralized and recentrifuged. This clear extract was used in the precipitin tests.

Anti-sera were prepared in rabbits. For direct precipitin reactions undiluted serum was run against the extract diluted from 1:10-1:8000. For absorption test undiluted serum was mixed with a heavy suspension of organisms. Enough were added to absorb completely the precipitin in the serum for the absorbing organisms. A grouping of the moniliae can be made by this test:

Group I—*M. albicans*, *M. psilosis*, and *M. candida* showed almost identical reactions. They showed cross precipitation in very high dilution, indicating that these strains contained similar antigens.

Group II—*M. parapsilosis* and *M. type 1* reacted alike, showing their similarity. Cross precipitations between the first group of moniliae and the second were noticed only in very low dilutions.

Group III—*M. krusei* did not seem to contain any precipitinogen similar to the other types.

Absorption tests showed a higher degree of specificity for each of the 3 groups. These 2 methods described, one biochemical and one serological, seem to show a definite correlation and give a simpler and more tangible means of differentiation of the species of moniliae.

## 7134

### Further Contributions to Methods of Barbitol Research.

THEODORE KOPPANYI, WILLIAM S. MURPHY AND STEPHEN KROP.

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In addition to procedures previously described,<sup>1</sup> we have adopted the principles of Myers' and Wardell's<sup>2</sup> blood cholesterol method for extraction of barbiturates.

A convenient amount of acidulated urine, blood (1 to 2 cc.), or well-ground, liquified tissues is pipetted into a small mortar containing sufficient Plaster of Paris to form a dry mixture, which is then

<sup>1</sup> Koppanyi, Murphy and Krop, *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1405.

<sup>2</sup> Myers and Wardell, *J. Biol. Chem.*, 1918, **36**, 147.

pulverized and transferred to a paper extraction shell. This shell is inserted into a perforated glass tube connected to a reflux condenser. Ten volumes of chloroform (10 to 20 cc.) are poured into the extraction flask, which in turn is heated to boiling on a water-bath for half an hour. The chloroform extract is then filtered and tested. If the test is negative the extract may be concentrated over a water-bath.

We have increased the sensitivity of our test previously described<sup>3</sup> by using cobalt acetate and, instead of the methyl alcohol solution of barium hydroxide, a 0.5% solution of lithium hydroxide in absolute methyl alcohol. Two or 3 drops of the 1% cobalt acetate and 0.5% lithium hydroxide reagents will produce a definite blue color in chloroform extracts of concentrations as low as 0.0025%. However, chloroform extracts can be concentrated 20 times, thus making the test sensitive to 1.25 parts per million.

We have described our test as colorimetric determination, but without the actual use of a colorimeter, because the colors produced by barium or other metallic hydroxides soon precipitate or fade if transferred to colorimeter cups. We have now succeeded in adopting the test for colorimetric readings with standard colorimeters. The procedure is as follows:

One cc. of the unknown chloroform extract is treated first with 0.05 cc. of a 1% cobalt acetate solution in absolute methyl alcohol and 0.3 cc. of a 5% (by volume) solution of iso-propyl amine (Research Laboratories, Eastman Kodak Co.) in absolute methyl alcohol. If barbiturates are present in the extract, a reddish-violet color develops, which is then compared in micro-cups with the color produced under the same conditions by barbitol solutions of known strength in chloroform. We found that a 0.025% solution of barbitol in chloroform usually suffices as a standard, against which unknown solutions over a wide range may be read. A simple formula, which may be used with any standard colorimeter, giving the number of milligrams of barbiturate per cc. of chloroform extract using the 0.025% solution as the standard is:

$$\frac{\text{Reading of standard} \times 0.25}{\text{Reading of Unknown}} = \text{Concentration of Unknown}$$

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<sup>3</sup> Koppányi, Murphy, and Krop, *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 542.

### Effect of Barbiturates in the Domestic Fowl.

THEODORE KOPPANYI AND WILLIAM S. MURPHY.

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Of 12 full grown fowls, 2 received 350 mg., 7 received 225 mg., and 3 were given 150 mg. sodium barbital per kg. by vein. Fowls receiving the minimum anesthetic dose, 225 mg. of sodium salt per kg. or over, never recovered to the extent that they were able to stand or walk. Within a period of one week they all died in barbital coma showing involvement of the respiratory system. The examination of the urine showed that they never excreted more than 33% of the dose injected and as a rule the elimination varied between 15% and 25%. The concentration in the urine was 1 mg. of barbital per cc. or less, and only in the fowl receiving 350 mg. of the sodium salt per kg. did it reach 2.5 mg. per cc. Dogs under similar circumstances have excreted 6 mg. per cc. of urine or more.

Fowls receiving 225 mg. per kg. of sodium barbital or more show a retention of the drug in the blood varying from 0.15 mg. to 0.05 mg. per cc. even 5 days following the administration of the drug. Similarly, one can detect the presence of barbital in the organs one week after the administration.

Two fowls were given 225 mg. of sodium barbital per kg. and one hour later 20 cc. of a 3% solution of uric acid was injected over a period of one hour. They showed the unprecedented diuresis of over 250 cc. of urine during a period of 18 hours. Yet they never recovered from the drug and excreted during a period of 7 days 21.1% and 24.5% respectively of barbital in the urine, showing a retention of the drug in the body.

Three chickens received 150 mg. of sodium barbital per kg.; no anesthesia was produced, but ataxia was present. Two of the fowls excreted 45% and 37.7% respectively of the drug during a period of 17 days, showed a slow recovery from ataxia, and even 24 hours after injection 0.15 mg. per cc. of barbital was present in the blood. Thus chickens excrete barbital for over a far longer period than mammals.

Seven chickens received intravenously 60 mg. of dial and neonal respectively; 100 mg. of phenobarbital, 25 mg. of nembutal, and 20 mg. of pernoston per kg. of body weight. They all recovered within 24 hours from the anesthesia, showing traces of these drugs in the

urine and none in the blood after that period, and, of course, they all survived.

Normal chickens, then, behave essentially as nephropathic mammals. They show a decreased elimination of barbital and retention of barbital in blood and organs and ultimate fatality caused by the drug if given in anesthetic doses. They all recover from the sleep produced by barbiturates other than barbital.

We performed several experiments to determine the cause of this remarkable phenomenon. We found that normal chickens all excreted, in one hour, over 65% of a total dose of 6.0 mg. of phenol-sulphonphthalein in one cc. injected intravenously, behaving in this respect exactly as mammals do. Chickens with 150 mg. of sodium barbital per kg. by vein excreted 50% of phenol red, whereas following 225 mg. they excreted 30% of the dye, or less. Therefore, in the fowl it would appear that barbital appreciably decreases kidney function as measured by the phenol red test.

It is also obvious from our data that fowls cannot concentrate barbital in the urine to the same extent as mammals.

A few preliminary experiments have been performed with turtles. The results show that these animals behave essentially like fowls. The remarkable behavior to barbiturates seems to be the same in all sauropsida.

## 7136

### Effect of Barbiturates in Experimental Nephrosis.

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We have shown<sup>1</sup> that bilateral nephrectomy greatly modifies the action of barbiturates in dogs; they do not recover from barbital, but do so from other barbiturates such as nembutal, pernoston, etc. As a further extension of this study, we used nephropathogenic agents to produce tubular lesions in mammals and investigated the action of barbiturates in animals with nephrosis. Dogs and rabbits were used for these experiments.

*A. Tartaric Acid.* Two dogs received 600 mg. of tartaric acid (neutralized with sodium carbonate) per kg. subcutaneously and

<sup>1</sup> Koppanyi, Murphy and Krop, *Arch. Int. de Pharm. et de Ther.*, 1933, **46**, 76.

subsequently given 225 mg. of sodium barbital per kg. by vein. The recovery from anesthesia was delayed, but the total excretion was but slightly subnormal (71-76%). After complete recovery and stoppage of excretion, the same animals, now showing evidence of grave tubular lesions, were again given 225 mg. of sodium barbital per kg. intravenously. They did not recover from the anesthesia and excreted less than 2% of the drug. Histological studies showed almost complete tubular disintegration, the glomeruli remaining fairly intact. Animals receiving 600 mg. of tartaric acid but no barbital do not become anesthetized and outlive the barbitalized animals. (See also Underhill, Wells and Goldschmidt.<sup>2</sup>)

*B. Uranium Acetate.* Three dogs and 2 rabbits were given 2 mg. uranium acetate in a 0.1% solution intravenously in divided doses over a period of 2 days. These animals did not recover from the depression produced by 225 mg. of sodium barbital per kg. by vein and excreted 41%, 38%, 8.7%, 2.3% and 1% of the drug respectively. The concentration of barbital in the urine was 1.0 mg. per cc., or less. Two dogs and one rabbit received in addition to uranium and sodium barbital, from 35 cc. to 50 cc. of a 10% glucose solution per kg. intravenously, excreting 56%, 38%, and 18% of the drug in the urine. Even though there appears to be an increase in the percentage of elimination in the diuretic animals, they never recovered from the barbital narcosis. The concentration of barbital in the urine was lower than in the non-diuretic animals. None of these animals, whether they were given glucose or not, ever recovered from barbital anesthesia and died within 114 hours following the administration of barbital. Three dogs similarly treated with uranium acetate, recovered within the usual periods from nembutal, pernoston, and neonal anesthesia.

*C. Potassium Chromate.* Four rabbits and one dog received 20 mg. of potassium chromate intramuscularly in divided doses over a period of 2 days and then were given intravenous injections of 225 mg. sodium barbital per kg. The 4 rabbits excreted only slightly subnormal amounts of the drug (from 50% to 75%); the dog, however, in which the chromate proved to be more destructive, excreted only slightly over 2%. The rabbits all recovered, but the dog died of barbital depression.

Four rabbits and one dog received, in addition to the doses of the former drugs, 50 cc. of a 10% glucose solution per kg. by vein. The rabbits recovered, excreting from 50% to 70% of barbital, the dog excreted only traces and died in barbital coma.

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<sup>2</sup> Underhill, Wells and Goldschmidt, *J. Exp. Med.*, 1913, 18, 317.

In all nephrotic animals receiving tartaric acid, uranium or potassium chromate and dying in barbital coma there was a retention of barbital in the blood until death, amounting to from 0.15 mg. to 0.05 mg. per cc. of blood.

In all animals the damage to the kidney function was checked by phenolsulphonphthalein elimination and subsequent histological examination.

*Conclusions.* In severe experimental nephrosis, dogs and rabbits behave with reference to barbiturates as bilaterally nephrectomized animals. They *never recover from barbital depression, remaining anesthetized until death, show retention of barbital in the blood, and also eliminate a relatively small percentage of the drug in the urine.* However, they do recover from the sleep produced by barbiturates other than those largely eliminated by the kidney. In less severe cases there is a retarded recovery and decreased elimination of barbital in the urine with no retention of the drug in the blood above normal.

## 7137 P

## Influence of a Certain Fraction of Pancreas Lipids on Carbohydrate Metabolism of Depancreatized Dogs.\*

A. G. EATON AND J. R. MURLIN.

*From the Department of Vital Economics, University of Rochester.*

Ever since the demonstration of the internal secretion of the pancreas there have been reports of substances antagonistic to insulin which increase the severity of diabetes. A blood sugar raising fraction of the pancreas reported by Gibbs, Root and Murlin<sup>1</sup> under the name of *glucagon* was precipitated by reagents which would precipitate the phospholipids and the cerebroside fractions of the pancreas fats. Further attention has been given recently to the separation of this fraction into its components. A fraction insoluble in acetone and ether, containing therefore the cerebroside and some sphingomyelin has given some striking effects on the D:N ratios and the R.Q.'s of depancreatized dogs, but no consistent effect

\* This paper was presented at the joint meeting of the Western New York branch and Section N of the A.A.A.S. at Syracuse, June 15, 1932.

<sup>1</sup> Gibbs, C. B. F., Root, E. W., and Murlin, J. R., *Quart. J. Exp. Physiol.* Suppl. vol., 1923, 125.

on blood sugar. While it does not at this time seem to be identical with the fraction called glucagon in the earlier report, the difference in effects may be due to a difference in dosage. For the time being it will be referred to simply as a diabetogenic fat fraction.

Depancreatized dogs were treated with this fraction by subcutaneous injection. The diabetogenic fraction after precipitation with ether was centrifuged out and suspended in alcohol. The alcohol was then poured into physiological saline and boiled until no odor of alcohol could be detected. Dosage was reckoned by the amount of pig pancreas from which the dose was obtained. With 3 dogs the D:N ratio was markedly increased as shown in Table I.

TABLE I.  
Summary of Results on Sugar and Nitrogen Excretion.

| Dog | Control 24 hours |       |      | Experimental 24 hours |       |      |          | % increase<br>excr. of<br>dextrose |
|-----|------------------|-------|------|-----------------------|-------|------|----------|------------------------------------|
|     | D                | N     | D:N  | D                     | N     | D:N  | Excess D |                                    |
| 14  | 29.56            | 12.12 | 2.44 | 34.74                 | 10.57 | 3.29 | 5.19     | 17                                 |
| 15  | 10.06            | 4.08  | 2.47 | 15.50                 | 4.41  | 3.51 | 5.44     | 54                                 |
| 17  | 6.36             | 5.61  | 1.13 | 17.61                 | 6.19  | 2.85 | 11.25    | 177                                |

The values given are for the 24 hours. The dogs were fed exactly the same on the 2 days. The urine was fractioned into three 4-hour periods and on one 12-hour period. The first 4-hour period (8 a. m. to 12 p. m.) was used as control, the diabetogenic fraction given just after the noon catheterization, and the effects on the metabolism studied in the afternoon. The dogs were fed at 8 p. m. The effect on sugar production and excretion was seen sometimes in the first

TABLE II.  
The Influence of Diabetogenic Fraction of Pancreas Lipids on Carbohydrate Metabolism of a Depancreatized Dog. Dog 16. Depancreatized 3/3/32. Last Insulin Injection 4/8/32.

| Time    | D/hr.                                                                                                                               | N/hr. | D:N   | April 13, 1932 |                        |                       |      |          |
|---------|-------------------------------------------------------------------------------------------------------------------------------------|-------|-------|----------------|------------------------|-----------------------|------|----------|
|         |                                                                                                                                     |       |       | Bl. Sugar      | L.CO <sub>2</sub> /hr. | L.O <sub>2</sub> /hr. | R.Q. | Cal./hr. |
| 8 a.m.  | 2.035                                                                                                                               | 0.558 | 3.644 | 255            |                        |                       |      |          |
| 9 "     | Injected subcutan. 30 cc. diabetogenic fraction of pancreas lipids in 0.85% NaCl, equivalent to approximately 350 gm. pig pancreas. |       |       |                |                        |                       |      |          |
| 10 "    |                                                                                                                                     |       |       |                | 6.01                   | 8.84                  | .70  | 41.41    |
| 11 "    |                                                                                                                                     |       |       |                | 5.51                   | 8.38                  | .68  | 39.28    |
| 12 noon | 1.42                                                                                                                                | 0.42  | 3.37  | 268            |                        |                       |      |          |
| 1 p.m.  |                                                                                                                                     |       |       |                | 5.86                   | 9.27                  | .66  | 43.44    |
| 2 "     |                                                                                                                                     |       |       |                | 5.28                   | 8.05                  | .67  | 39.28    |
| 3 "     |                                                                                                                                     |       |       |                | 4.72                   | 7.40                  | .66  | 34.69    |
|         |                                                                                                                                     |       |       |                |                        | Average               | .68  | 39.62    |
| 4 "     | 1.25                                                                                                                                | 0.47  | 2.63  | 255            |                        |                       |      |          |
| 8 "     | 0.71                                                                                                                                | 0.34  | 2.06  | 253            |                        |                       |      |          |
|         |                                                                                                                                     |       |       | April 14, 1932 |                        |                       |      |          |
| 8 a.m.  | 2.97                                                                                                                                | 0.83  | 3.60  | 250            |                        |                       |      |          |

experimental 4-hr. period, sometimes more plainly in the second, and always in the night period following feeding at 8 p. m.

The one dog which did not respond with a marked increase of D:N ratio, for the 24 hours, nevertheless showed a large increase of sugar excretion in the night period following injection at noon. This dog gave typical respiratory quotients for complete pancreatic diabetes on 2 different days and, as shown in Table II, exhibited a delayed depression of the R.Q., beginning 3 hours after administration of the fat fraction. The R.Q. was depressed as much or more than this in one other depancreatized dog and in a castrated but otherwise normal dog. Since depancreatized dogs exhibit very little ketosis, the depression of the R.Q. below the diabetic level is suggestive of gluconeogenesis from fatty acids.<sup>2</sup> We have not found any report in the literature of a similar effect from a similar lipid fraction.

## 7138 C

### Dinitrophenol Hyperglycemia. I. Its Independence of Asphyxia.\*

V. E. HALL, C. A. BROWN AND M. SAHYUN. (Introduced by E. G. Martin.)

*From the Laboratory of Physiology, Stanford University.*

The fact that 1-2-4 dinitrophenol, which greatly accelerates the oxidative metabolism of tissues, increases blood sugar concentration has been reported by Magne, Mayer and Plantefol<sup>1</sup> and confirmed by Hall, Field, Sahyun, Cutting and Tainter.<sup>2</sup> Since the large increase in oxygen usage of the tissues provoked by the drug might readily outstrip the ability of the respiratory and circulatory mechanisms to deliver oxygen and so lead to general bodily asphyxia, it seemed possible that the hyperglycemia might be of such asphyxial origin. The experiments described herein were designed to test this hypothesis.

Cats, anesthetized with pentobarbital, received doses of 15 or

<sup>2</sup> Hawley, Estelle E., Johnson, Carroll, and Murlin, J. R., *J. Nutrition*, 1933, **6**, 523.

\* Supported in part by the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

<sup>1</sup> Magne, H., Mayer, A., and Plantefol, L., *Ann. Physiol. Physicochim. biol.*, 1932, **8**, 1.

<sup>2</sup> Hall, V. E., Field, J., Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.*, 1933, **106**, 432.

20 mg. per kilo of 1-2-4 dinitrophenol intramuscularly in the form of its sodium salt. Blood samples at half-hour intervals were analyzed for sugar by the method of Sahyun,<sup>3</sup> for oxygen and total carbon dioxide content by the manometric method of Van Slyke and Neill,<sup>4</sup> and for plasma pH by the colorimetric method of Hawkins.<sup>5</sup> Five experiments with 20 mg. per kilo, 2 with 15 mg. per kilo and 4 controls without any drug were performed. Within each group, the experiments agreed with each other satisfactorily. The results in animals with the higher dose and in the controls are presented in Table I. The animals receiving 15 mg. per kilo gave values intermediate between those of the other groups.

TABLE I.  
Average Arterial Blood and Other Changes in Dinitrophenol Intoxication.

|                       | Controls |       |       |        | "DNP," 20 mg./kg. |       |       |        |
|-----------------------|----------|-------|-------|--------|-------------------|-------|-------|--------|
|                       | Initial  | ½ hr. | 1 hr. | 1½ hr. | Initial           | ½ hr. | 1 hr. | 1½ hr. |
| Glucose mg. %         | 98       | 110   | 125   | 146    | 102               | 164   | 211   | 220    |
| Oxygen vol. %         | 17.2     | 17    | 17.2  | 18     | 18.8              | 18.8  | 19    | 19.2   |
| Carbon dioxide vol. % | 25.2     | 20.2  | 22    | 22     | 21.2              | 17.8  | 17.4  | 16.2   |
| Plasma pH             | 7.3      | 7.32  | 7.37  | 7.39   | 7.4               | 7.46  | 7.48  | 7.49   |
| Rectal temp. deg. C.  | 37.4     | 37.7  | 37.7  | 37.5   | 37.4              | 37.7  | 39    | 40.1   |
| Resp. rate per min.   | 21       | 26    | 25    | 26     | 27                | 37    | 50    | 103    |
| Arterial pr. mm. Hg.  | 136      | 127   | 111   | 100    | 137               | 121   | 117   | 115    |

From our results there is no indication of the development of asphyxia under the influence of dinitrophenol; the oxygen content of the blood is unchanged, the total carbon dioxide content falls, while the plasma pH rises. The findings thus support our<sup>2</sup> earlier conclusion that, at least before the terminal collapse sets in, the drug produces neither anoxemia, asphyxia nor acidosis. They are also in agreement with the determinations of plasma pH made by DeEds,<sup>6</sup> employing the glass electrode. The tendency to acapnia and alkalosis, which has appeared in these experiments, is no greater in the experiments with the drug than in the controls. The fact that the usual metabolic response was present and vigorous is attested by the marked increases in rectal temperature and respiratory rate.

In spite of the absence of asphyxia, there developed a marked

<sup>3</sup> Sahyun, M., *J. Biol. Chem.*, 1931, **93**, 227.

<sup>4</sup> Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **41**, 523.

<sup>5</sup> Hawkins, J. A., *J. Biol. Chem.*, 1923, **57**, 493.

<sup>6</sup> DeEds, F., *Science*, 1933, in press.

hyperglycemia, the rise in blood sugar being approximately 3 times as great as that in the controls. The difference of the means of the final blood sugar values between the dinitrophenol animals and controls was 73.85 mg. per 100 cc. Since this difference exceeds its own probable error by 4.4 times, it is statistically significant.

A possible cause for the rise in blood sugar might be the development of a cerebral anemia. Since the general arterial pressure, on which the cerebral blood flow rate is primarily dependent,<sup>7</sup> declined to a smaller degree in the experiments with the drug than in the controls, the cerebral circulation is presumably at least as adequate in the former as in the latter. This conclusion assumes that no significant cerebral vasoconstriction has occurred. However, since dinitrophenol causes a great increase in cardiac output without much change in arterial pressure,<sup>2</sup> a marked general vasodilatation must occur. This is supported by the observation of Markee<sup>8</sup> that the drug causes a flushing of the uterine transplant in the anterior chamber of the eye. While the occurrence of general vasodilatation is no proof of a local cerebral vasodilatation, vasomotor adjustments tend to favor cerebral blood flow.<sup>9</sup> Accordingly we feel that the occurrence of cerebral vasoconstriction under the influence of the drug is improbable. The above considerations render it unlikely that cerebral anemia was the cause of the blood sugar rise.

We conclude that the hyperglycemia produced by dinitrophenol is not secondary to a general bodily asphyxia, as is the case with that produced by many other drugs. The possibility that a local cellular anoxemia or acidosis of the sympathetic glycogenolytic centers of the brain stem may be the mechanism of this hyperglycemia is now under investigation.

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<sup>7</sup> Wiggers, C. J., *Circulation in Health and Disease*. 2nd Ed., P. 155. Philadelphia, 1923.

<sup>8</sup> Markee, J. E., personal communication.

<sup>9</sup> Bronk, D. W., and Gesell, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1926, **24**, 257.

### Nuclear Changes Following Intravenous Injection of Various Solutions.\*

JACK LEE. (Introduced by E. V. Cowdry.)

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St. Louis.*

Opinions differ as to whether intranuclear inclusions are pathognomonic of virus diseases. Thus, Cole and Kuttner<sup>1</sup> expressed the view that when typical intranuclear inclusions are found, the presence of a filterable virus is to be assumed unless its absence can be proved experimentally, while Cowdry<sup>2</sup> stated that the presence of intranuclear inclusions should not be taken at face value as indicating the action of some filterable virus. Cowdry<sup>3</sup> also entertained the possibility that the inclusions may be produced artificially without viruses. Heinbecker and O'Leary<sup>4</sup> described the appearance of bodies simulating intranuclear inclusions in nerve cells after electrical stimulation<sup>5</sup> and Davenport, Ranson and Terwilliger<sup>6</sup> produced similar structures by immersion of ganglia in hypertonic salt solutions.

Cats were injected through the femoral vein, under ether anesthesia, with glucose, sodium chloride, sodium bicarbonate, distilled water, or Salyrgan. At various intervals following the injections, the animals were killed by exsanguination, and material was fixed in Zenker's fluid containing 5% acetic acid, and stained with hematoxylin and eosin, erythrosin and azur I.

(a) *Glucose.* When 60 cc. of 50% glucose per kilo of body weight was injected slowly during 1 hour and the animal killed immediately, marked changes were observed in the nuclei of spinal ganglion, anterior horn and Purkinje cells. The nuclear chromatin, dispersed in reticular fashion in the cells of normal controls, formed

\* Aided by a grant from the Rockefeller Foundation for research in virus diseases.

<sup>1</sup> Cole, R., and Kuttner, A. G., *J. Exp. Med.*, 1926, **44**, 855.

<sup>2</sup> Cowdry, E. V., *Arch. Path.*, 1930, **10**, 23.

<sup>3</sup> Cowdry, E. V., in Rivers' "Filterable Viruses." Baltimore, Williams and Wilkins, 1928, 428.

<sup>4</sup> Heinbecker, P., and O'Leary, J. L., *Anat. Rec.*, 1930, **45**, 219.

<sup>5</sup> In a personal communication Heinbecker and O'Leary express doubt as to the validity of their original interpretation and are inclined to attribute their results to evaporation occurring during *in vitro* stimulation.

<sup>6</sup> Davenport, H. A., Ranson, S. W., and Terwilliger, E. H., *Anat. Rec.*, 1931, **48**, 251.

a compact mass about the nucleolus and was separated from the nuclear membrane by a halo. The nucleolus was noticeably vacuolated and swollen. The only cytoplasmic alteration noted was in the spinal ganglion cells; here a varying degree of central chromatolysis was apparent. When animals of like weight were killed 3 hours after the injection of a similar quantity of glucose, the nuclear change was much less obvious, indicating a return to normal.

(b) *Sodium chloride*. 150 cc. of 30% sodium chloride was injected over a period of 45 minutes and the 3 animals killed immediately. Purkinje, anterior horn and spinal ganglion cells showed changes similar to those seen in glucose injected animals; but the chromatin was more acidophilic and less concentrated about the nucleolus.

(c) *Sodium bicarbonate*. After injection into 3 animals of 50 cc. of a 20% solution of sodium bicarbonate, masses were discovered in the nuclei of spinal ganglion cells very similar to those observed in glucose injected animals.

(d) *Distilled water*. In 4 animals killed following the injection of from 300 to 400 cc. of distilled water in 1 hour and 15 minutes, intranuclear bodies were observed which simulated those described by Covell<sup>7</sup> in the nerve cells of acute experimental poliomyelitis. The nuclei of the water injected animals, in contrast to those containing Covell's inclusions, showed no evidence of margination of basophilic chromatin, or disappearance or conversion of acidophilic chromatin.

(e) *Salyrgran*. This pronounced diuretic was injected intramuscularly into 6 cats in  $\frac{1}{2}$  cc. amounts daily for 4 days, and intravenously into 4 cats in 1 cc. doses daily for 5 days. The animals were sacrificed at graded intervals. Nuclear changes were observed in all of the first group, but in none of the second, nor in 35 control cats not subjected to this treatment but employed in the laboratory for other experiments. They occurred in the pancreas of 6, kidneys of 3, testes of 2, and adrenals of 2; were absent in the nervous system and were not noted in these organs after treatment with glucose, sodium chloride, bicarbonate or distilled water. The nuclear alterations were rather localized, but so conspicuous in these 6 cats that they were seen almost immediately. They presented considerable diversity. Some of them were characterized by the margination of basophilic chromatin on the nuclear membrane and the appearance of acidophilic bodies separated from the nuclear mem-

<sup>7</sup> Covell, W. P., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 927.

brane by a halo. The nuclei were hypertrophied to a maximum of twice their normal diameter. There was some accompanying cytoplasmic degeneration, mitotic division and regeneration of acini. The nuclear alterations persisted as long as a month after the last injection.

It is not claimed that any of the changes are identical with those caused by viruses though they resemble them in certain particulars. The experiments do show, however, that nuclear alterations something like those previously reported *in vitro* may be produced *in vivo* by relatively mild procedures which do not kill the animals. They also raise several questions for further study.

## 7140 C

### Bronchodilator Activity of Tyramine and its N-Methyl Derivatives.\*

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There are few reported quantitative comparisons of the relative pressor and bronchodilator effects of chemicals in the epinephrine series, and it is an open question whether certain structural changes in such compounds may result in a relative increase of bronchodilator activity without a corresponding increase in pressor activity.

Tyramine, an extensively studied compound of this type, is a comparatively good pressor agent, but Jackson<sup>1</sup> reports that it does not exert any bronchodilator activity. He found, however, that horde-nine, the N-dimethyl derivative of tyramine, dilates the bronchi, although it exerts considerably less pressor activity. Thus, with these compounds there seems to be a differentiation between pressor and bronchodilator effects which is compatible with the evidence of Cannon and Rosenblueth<sup>2</sup> that the chemical mediators involved in the transmission of sympathetic stimulation to the bronchi and the blood vessels are not the same.

In the present work, a comparative study of the pressor and bronchodilator activities of the following compounds was made upon a series of 6 dogs and 4 cats, following technique already described.<sup>3</sup>

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\* Supported in part by the Christine Breon Fund for Medical Research.

<sup>1</sup> Jackson, D. E., *J. Pharm. and Exp. Therap.*, 1913, **5**, 479.

<sup>2</sup> Cannon, W. B., and Rosenblueth, A., *Am. J. Physiol.*, 1933, **104**, 557.

<sup>3</sup> Alles, G. A., and Prinzmetal, M., *J. Pharm. and Exp. Ther.*, 1933, **48**, 161.

1.  $\beta$ -4-Hydroxyphenylethyl amine (Tyramine).
2.  $\beta$ -4-Hydroxyphenylethyl methylamine (Methyl tyramine).
3.  $\beta$ -4-Hydroxyphenylethyl dimethylamine (Hordenine).
4.  $\beta$ -4-Hydroxyphenylethyl trimethyl ammonium iodide (Hordenine methiodide).

It was noted that, contrary to the finding of Jackson and others, tyramine actively dilates constricted bronchi in suitable preparations. Methyl tyramine is equally effective both as a pressor and bronchodilator and in some cases appears to be somewhat the more active. These effects were observed with doses as low as 0.001 millimols per kilogram, injected intravenously. Equimolecular dosage of hordenine is regularly without action on blood pressure or the bronchi but 5 to 10 times this dose usually gives responses comparable to those of tyramine. Since hordenine exerts an effect similar to that of nicotine, it probably acts through a different mechanism than tyramine which has no nicotine-like action. Likewise with hordenine methiodide, nicotine-like pressor and bronchodilator effects are readily observed and this compound is about 50 times more active than hordenine itself in both its pressor and bronchodilator activities. When hordenine and hordenine methiodide are injected into unphithed animals in equally effective doses with regard to pressor effects they produce equivalent stimulation of the respiration, thereby indicating also a close similarity in their nicotine-like stimulation of the central nervous system.

From the present work there seems to be no differentiation of relative pressor and bronchodilator activities in the series of compounds here studied, although a differentiation of the mechanism by which they produce these actions is indicated by a comparison of tyramine or its methyl derivative with hordenine or its methiodide.

## 7141 P

### Comparison of Osteogenic Power of Periosteal Transplants from Rib and Tibia.

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The object of this study was to compare the osteogenic power of periosteal transplants of the rib with those of the tibia.

Six healthy adult dogs were employed. All operations were car-

ried out with aseptic technique under ether anesthesia. In each of 3 dogs (Group I) equal-sized strips of periosteum, free from cortical bone, were removed from the mid-portion of the right 7th rib and from the middle one-third of the shaft of the right tibia. Both transplants were sutured on the external surface of the tibialis anticus muscle fascia. In each of 3 dogs (Group II) equal-sized pedicle flaps of periosteum from the rib and tibia were made. Their free ends, which were about 3 cm. long, were turned laterally and sutured on to the intercostal and tibialis anticus muscle fascia, respectively. Two of the animals in the latter group were discarded because of infection of the wounds.

Seven weeks after operation one animal of Group I was sacrificed and an autopsy was performed. In this animal microscopic examination of the transplant of periosteum from the tibia showed no evidence of osteogenesis; the transplant of periosteum from the rib, on the other hand, showed several areas with formation of new intramembranous bone. Autopsies were performed on the remaining 3 animals (2 of Group I and one of Group II) 8 months after operation. The findings in the specimens of each of these animals were essentially the same. Gross examination showed solid pieces of bone in place of each of the transplants of rib periosteum. The transplants of periosteum from the tibia felt tough and fibrous but they were pliable and showed no gross evidence of bone formation. In each case, microscopic examination of the periosteum from the rib showed well formed cortical and medullary bone, with marrow tissue and active osteogenesis especially at the periphery. The specimen of the pedicle graft of rib periosteum showed approximately 25% greater amount of new bone and also more active osteogenesis, than the specimen of free graft of rib periosteum. The grafts of periosteum of the tibia showed nothing except fibrosis of the connective tissues. In one of the latter sections there was a small amount of calcification but no evidence of ossification.

These experiments emphasize for the first time that a great difference may exist as to the osteogenic power of *periosteum* from 2 bones in the same animal. They confirm the findings (Ollier,<sup>1</sup> Burman,<sup>2</sup> Haldeman<sup>3</sup>) that periosteal transplants, free from adhering cortical bone, may have the power to form new bone; also, they help to explain the cause for the superiority of full-thickness grafts (cor-

<sup>1</sup> Ollier, L., *J. de Physiol. de l'Homme et des Animaux*, 1859, **2**, 1; 1863, **6**, 517.

<sup>2</sup> Burman, M. S., and Umansky, M., *J. Bone and Joint Surg.*, 1930, **12**, 579.

<sup>3</sup> Haldeman, K. O., *Arch. Surg.*, 1932, **24**, 440; *J. Bone and Joint Surg.*, 1933,

tical bone and periosteum) of the rib as used by Eloesser,<sup>4</sup> Whitman,<sup>5</sup> Kleinberg,<sup>6</sup> and Bisgard.<sup>7</sup>

## 7142 C

## Certain Properties of Bacterial Mucus.

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It has previously been reported that certain strains of *B. subtilis* exhibit a peculiar phenomenon when grown on saccharose agar plates.<sup>1</sup> The colonies are stringy and contain besides the bacteria a large amount of apparently amorphous, mucoid material. This material under appropriate conditions spreads out from the colonies on the surface of the agar for a distance of 10 to 20 mm. forming a halo around the colonies. Bacteria cannot be found in the halo and transplants made from it do not grow on the usual agar plates or in broth. However, when the amorphous substance is transplanted to saccharose agar plates it grows in tiny transparent colonies. The detailed study of this phenomenon will be described in another place<sup>2</sup> and only a few properties of it will be mentioned here.

It has been possible to make only 3 consecutive transfers of the amorphous substance. The bacteria are not regenerated in these cultures. In the developing colonies of the bacterium the amorphous substance usually appears first as a capsule around a few of the bacteria which often appear swollen and disintegrating. Similar phenomena, although varying quantitatively in degree, may be observed in cultures of many different types of bacteria, but are usually seen only in the first few transplants after isolation.

It is known that under certain conditions various bacteria produce large amounts of mucoid substances. "Ropy beer" is a well known example of this. This phenomenon has been attributed to an excessive production of bacterial capsules. According to our obser-

<sup>4</sup> Eloesser, L., *Arch. Surg.*, 1920, **1**, 428.

<sup>5</sup> Whitman, A., *Am. J. Surg.*, 1929, **6**, 801.

<sup>6</sup> Kleinberg, S., *J. Bone and Joint Surg.*, 1929, **11**, 66.

<sup>7</sup> Bisgard, J. D., *Arch. Surg.*, 1933, **26**, 796.

<sup>1</sup> Dienes, L., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 1205.

<sup>2</sup> Dienes, L., *Zbl. f. Bakt. I. O.*, in press.

vations the mucoid substance grows not only as a capsule around the bacterium but is also produced independently from the bacteria in the medium.

We tried to determine with several different methods whether the mucoid substance contains morphological elements which would account for these surprising growth phenomena. In many cultures, using dark field illumination or in dark background preparations as described in the previous note, the amorphous substance seems to consist of tiny granules. In other cultures, however, these granules were absent. The application of flagella staining methods to our cultures resulted in two observations of interest. (1) In certain bacilli 2 to 10 or more small round bodies are formed which later are liberated and sometimes are found in great numbers between the intact bacteria. These small bodies are certainly different from the previously mentioned ultramicroscopic granules and their significance is unknown. (2) Especially in young cultures, the seemingly amorphous mucoid substance consists of long fine interwoven filaments as shown in Fig. 1. Preparations from liquid cultures developing a stringy consistency show masses of these filaments. In a few cases we succeeded in demonstrating these structures in a young halo developing around *subtilis* colonies. With certain staining methods the bacteria in these cultures appear encapsulated but in preparations stained successfully by flagella staining methods the capsule resolves into a mass of fine filaments.

After these observations were made with *subtilis* strains we studied other bacteria including meningococci, gonococci, pneumococci, streptococci producing a mucoid growth, and influenza bacilli using flagella staining methods. The organisms were studied immediately after isolation from clinical material. In the case of all organisms mentioned above, the cultures contained masses of the filamentous structures. Fig. 3 shows the filaments from a culture of meningococci and Fig. 5 shows Type III pneumococci with capsules. In these cases we have not observed the growth of the filaments independently from the bacteria.

Similar filamentous structures were seen by several authors and Hinterberger described them exactly<sup>3</sup> in mucoid cultures of the anthrax bacillus and later in pyocyanous cultures.<sup>4</sup> Zettnow observed them accidentally in the cultures of many bacteria.<sup>5</sup>

The most important question in connection with these filaments

<sup>3</sup> Hinterberger, A., *Zbl. f. Bakt. I. O.*, 1901, **30**, 417.

<sup>4</sup> Hinterberger, A., and Reitmann, C., *Zbl. f. Bakt. I. O.*, 1904, **37**, 160.

<sup>5</sup> Zettnow, E., *Zschs. f. Hyg. u. Inf. Kr.*, 1918, **86**, 25.

Fig. 1.

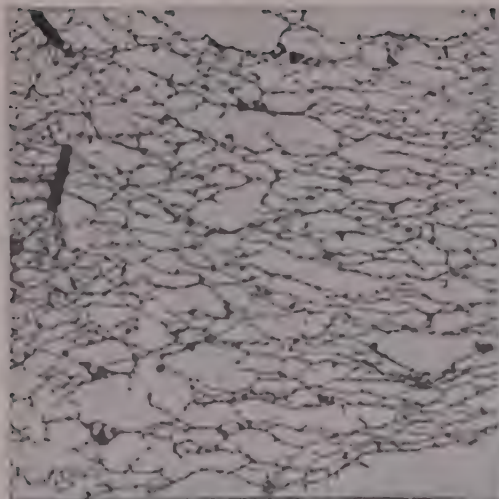


Fig. 2.



Fig. 3.

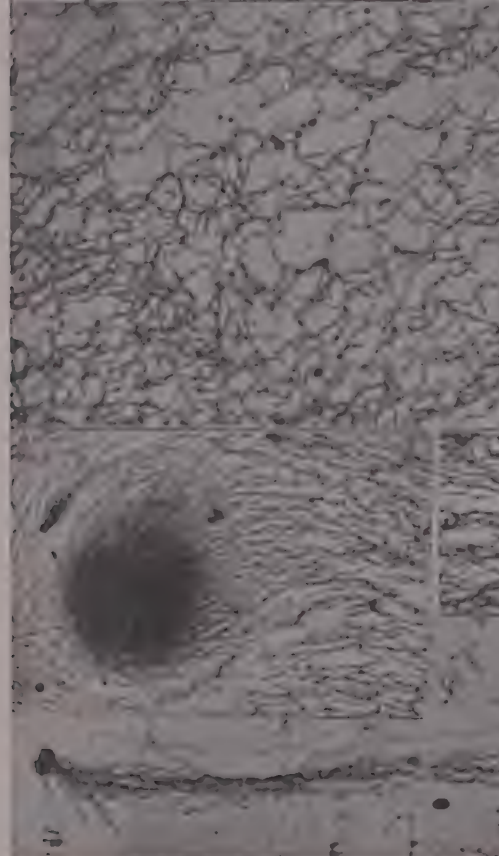


Fig. 4.

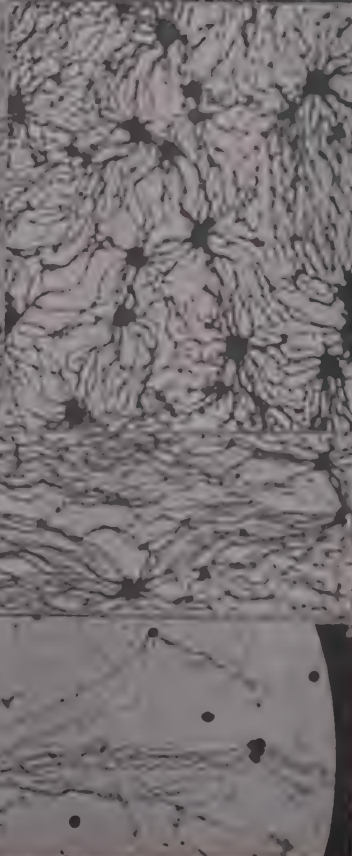


Fig. 5.

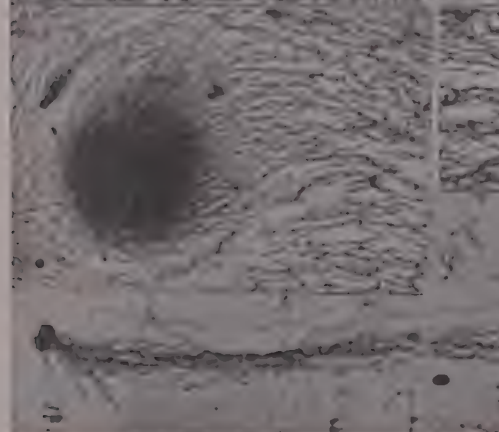


Fig. 6.

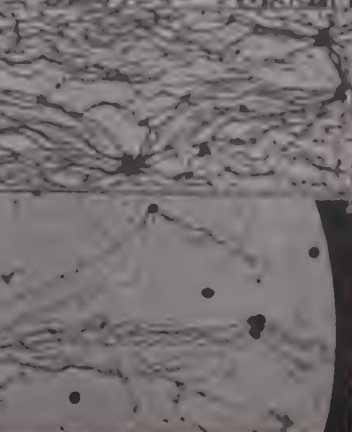
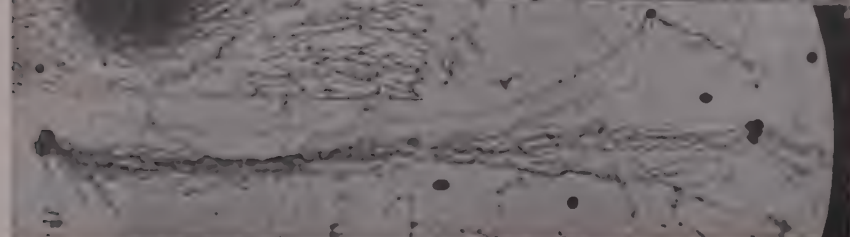


Fig. 7.



All pictures are taken with the same magnification,  $1 \times 1000$ .

FIG. 1. Fresh culture of *B. subtilis* strain 9 on saccharose agar.

FIG. 2. The same bacillus from saccharose broth culture.

FIG. 3. Meningococci, saccharose agar. Second generation from spinal fluid.

FIG. 4. A Gram-negative bacillus, 3247, on saccharose agar.

FIG. 5. Pneumococcus type III rabbit serum saccharose agar.

FIG. 6. The Gram-negative bacillus 3247 from a ropy saccharose broth culture.

FIG. 7. The same bacillus from saccharose broth culture with long filaments.

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is whether they represent structures actually present in the cultures or whether they are artefacts in the preparations. The evidence now at hand strongly suggests the former. They differ from artefacts which we see in other preparations of this type. Attempts to obtain similar structures from such substances as glue, gelatin, or a stringy solution of purified mucin were unsuccessful. On the other hand in preparations of spinal fluid from cases of meningitis and from sputum containing numerous bacteria it was possible to observe the same filamentous structures as found in the cultures. In preparations from spinal fluid the fibrin from a freshly formed coagulum appears in the form of long interwoven filaments which furnishes additional evidence that these structures also in the bacterium cultures are genuine. In preparations from bacterial cultures the filaments are often torn and ruptured as a result of smearing, indicating that the filaments were present in the liquid before it dried on the cover slip. It is also significant that the filamentous structures are characteristically different in preparations made from cultures of different bacteria whether grown on solid or in liquid media. Figs. 1 and 2 show the filaments from agar and broth cultures of *B. subtilis*. Figs. 4, 6, and 7 illustrate the same structures from similar cultures of a gram negative bacillus. The difference in the type of filaments produced by the 2 organisms is apparent. Artefacts usually vary in appearance and do not reproduce the same characteristic pictures as do these structures.

It would be early to express an opinion concerning the nature of the filaments and as to whether they are living structures or not. The formation of the halo and the development of tiny colonies in transplants from it strongly suggest growth phenomena. The possibility of the independent growth of the capsular substances which play such an important rôle in virulence and immunity gives a special interest to the study of the described filamentous structures.

*Summary.* In the cultures of numerous bacteria, and particularly in recently isolated strains, masses of fine filaments are present which are seen only in preparations stained with flagella stains. In stringy

broth cultures similar filamentous structures are present. The capsule is frequently seen to consist of a thick agglomeration of these filaments around the bacteria. In certain subtilis cultures the mucoid substance which consists largely of these filaments shows phenomena suggesting that it is capable of growth independently from the bacteria.

Notes on the staining methods: The filaments could be stained with all flagellar staining methods tried. The following technique gave most constantly satisfactory results. The agar or broth cultures are spread on a cover slip with the help of a small drop of water without being previously suspended or diluted and quickly dried. It is sometimes helpful to extract the preparation after drying with distilled water. For a mordant, Loeffler's solution is used (prepared a few weeks previously) and is applied for 2 to 3 minutes without heating. The preparation is then silvered with aethylamin silver solution. Not all samples of the mordant give satisfactory results.

## 7143 C

### Kidney Function in Pregnancy. II. Effect of Posture on Diuresis.

ELISABETH W. WALKER, MARY McMANUS AND JAMES C. JANNEY.  
(Introduced by A. W. Rowe.)

*From the Evans Memorial, Massachusetts Memorial Hospitals, and the Florence Crittenton Home, and the Boston University School of Medicine.*

In line with observations on normal non-pregnant individuals as to the influence of posture on urine volume,<sup>1</sup> an opportunity has presented itself to carry out similar studies during pregnancy. One private patient volunteered to carry out a series of tests throughout her pregnancy. Commencing about 20 weeks before term, she did 4 tests per month until the time of delivery, and 2 series postpartum.

The tests were done in 4 positions, the horizontal back position, sitting up, the Trendelenberg position, and horizontal on the side. In the latter position the patient was allowed to turn on either side at will. Each monthly series included these 4 tests, and, so far as possible, the tests were arranged for every other day.

Physically Mrs. J. H. was a primipara in excellent health until the very end of her pregnancy. She was not subjected to any ex-

<sup>1</sup> Janney, J. C., Riley, G., and Walker, E. W., *Proc. Soc. Exp. Biol. and Med.*, 1933, **31**, 398.

tensive kidney study. She had the usual prenatal care, with checking of the blood pressure, and urine, and all toxic symptoms, but it was felt to be hardly necessary to study her further as the chief question was not of her conformance to an average standard but of the comparison in a single patient of water diuresis under 4 different conditions. The arrangement of the tests (every other day in each series of four) was such that much change during the course of a series was, so far as possible, minimized. Consequently we feel that the conditions are nearly enough uniform to justify comparison. The patient's health continued good until the week before her delivery when her pressure began to creep up and she showed a very slight trace to a slight trace of albumen in the urine. Between the time of the next to the last test, on 4/18, and the last, on 4/20, her systolic pressure had jumped 16 points to 148. She was then 10 days before her calculated full term, and the day after the last test to avoid further risk labor was induced by rupture of the membranes. It will therefore be noted that in Table I and Fig. 1, which show the data on this patient, the weeks antepartum and postpartum are calculated from the time of actual delivery which is about 10 days ahead of the date figured from the last catamenia.

TABLE I.

Showing Urine Volumes of Mrs. J. H. at Various Stages of Pregnancy and Puerperum in the Several Postures of the Test.

|               | 18   | Weeks Antepartum |      |      |      | Postpartum |      |
|---------------|------|------------------|------|------|------|------------|------|
|               |      | 14               | 9    | 5    | 2    | 3          | 9    |
| Recumb. back  | 1305 | 1342             | 767  | 828  | 738  | 1113       | 1135 |
| Sitting       | 1059 | 898              | 1252 | 699  | 438  | 879        | 537  |
| Trendelenberg | 990  | 1313             | 994  | 1180 | 888  |            | 1039 |
| Recumb. side  | 871  | 1355             | 1317 | 1148 | 1215 | 1000       | 1164 |
|               |      |                  |      |      |      | Nursing    |      |

Comparison of her horizontal back elimination curves with the experience reported for normal pregnant women<sup>2</sup> shows that she conforms to the average trend of these other patients, but runs below the average amount from the 10th week antepartum on. As pregnancy advances the response to the test in the sitting position becomes progressively worse, both actually and compared with the horizontal back tests, and that, in the last sitting test, 2 weeks before the induction of labor, the volume has dropped to 32% of her normal response, whereas the last antepartum test in the horizontal back position, done within 2 days of the one last mentioned, has declined only to 55% of the normal. The tests in the Trendelenberg

<sup>2</sup> Janney, J. C., and Walker, E. W., *J. Am. Med. Assn.*, 1932, **99**, 2078.

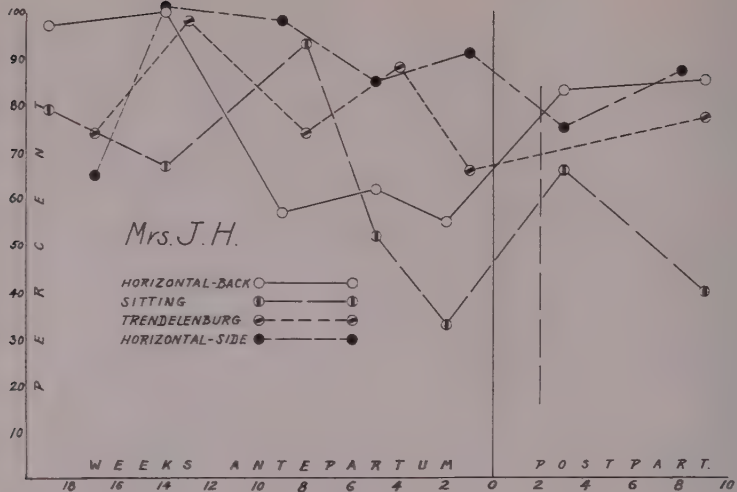


Fig. 1.

The output curves of Mrs. J. H. from the 18th week antepartum on in the several positions of the test. The calculation of the curves is explained in the text.

position show less drop as pregnancy progresses than those in either the horizontal back or the sitting postures. The last test in Trendelenberg, antepartum, showed a volume of 66% compared to the horizontal of 55%. The most interesting curve of the 4 is that in the horizontal side position, which held up to nearer a normal non-pregnant level than any of the others, and on the last test antepartum showed an output of 91%.

The tests done approximately 2 weeks postpartum are somewhat contradictory. The horizontal back test (84%) and the sitting test (66%) showed marked recovery from their antepartum levels, suggesting that the loss of function antepartum was largely due to mechanical obstruction, and was influenced by posture, and that it disappears rapidly when delivery relieves this obstruction. The horizontal side response (75%) was lower than the 91% which was the last antepartum figure, suggesting that beside the mechanical obstruction, which is largely relieved in this position, even during the antepartum tests, there is some other factor involved. It seems not unreasonable to suppose that antepartum there is a certain amount of tissue stored water, occult edema, and that once back pressure on the kidneys is released by the lateral horizontal position this fluid begins to augment the fluid ingested. At 8 weeks postpartum the results of all tests, except in the sitting position, range

between 77 and 87%. The sitting test shows a decided drop, almost back to the lowest antepartum figure, which is entirely unexplained.

The computation of these figures assumes the highest test in the horizontal back series as 100%. This figure is 1342 cc., thoroughly normal when compared to the averages of the normal non-pregnant patients studied in the past. The horizontal back position was the one used in previous tests, with both pregnant and non-pregnant subjects, and the other postures used in this and a former paper are essentially comparisons of water diuresis in different positions with the standard horizontal back position. All other tests, therefore, are related by percentage to this 100% figure.

On theoretical considerations these curves are just what we should expect. The mechanical interference with urine output is increased when the patient sits up and, in addition to the pressure caused by its volume, the weight of the uterus and its contents rests on the ureters at the brim of the pelvis. These effects do not make their appearance, however, until the latter half of pregnancy either in the X-ray or in the functional disturbance. This corresponds to anatomical fact, for until this time the uterus is hardly large enough to cause pressure at the brim of the pelvis. When the test is performed in the Trendelenberg position there is a tendency for gravity to take the weight off the pelvic brim and the ureters, but, mechanically, as the results show, this is not as efficient in relieving the pressure as lying in the side. With the patient in Trendelenberg there is some pressure on both ureters though not so much as in the horizontal position. With the patient on her side the tendency is to relieve the pressure on both ureters. It might be supposed that the upper ureter was the only one relieved, but as pregnancy advances and the uterus becomes larger its center of gravity moves further forward until in the side position the tendency is for it to roll out of the abdomen altogether and not just rest on the lower side. It is this mechanical relation which relieves the lower as well as the upper ureter. If it were possible to accomplish we should expect better diuresis in the knee chest position or with the patient on all fours than in any other posture, but these positions cannot be tolerated long enough for the accomplishment of this test, much less for any therapeutic results. It is an attractive supposition that this 4-footed posture may be an important factor in the absence of eclampsia in animals.

Despairing of finding another patient who would cooperate so intelligently as the one here reported we turned to the next best method of substantiating these figures, namely, the testing of a

number of patients with one series of tests during the last month of their pregnancy. In this series 3 tests were done, in the horizontal back, the sitting, and the horizontal side positions respectively. The subjects of these tests were patients at the Florence Crittenton Home in Boston. The tests were arranged so that they were done on consecutive days with each subject. There were 12 subjects tested and of these 11 were clinically normal at all times. One patient a few days subsequent to her tests showed a blood pressure of 154/100 and headache of 2 days' duration. The urine was negative. She had none of these symptoms, however, during the time of her tests. All of the subjects were tested during the last month of pregnancy. The longest time before delivery that any series was finished was 26 days and the shortest 4 days. In all the subjects average of the tests was 17 days antepartum. No tests were done postpartum. The results of these tests are given in Table II and Fig. 2.

TABLE II.  
Showing Urine Volumes of Florence Crittenton Patients in the Several Positions of the Test.

| Name        | Horizontal Back | Sitting | Horizontal Side | Dates of Tests   | Delivery |
|-------------|-----------------|---------|-----------------|------------------|----------|
| Eleanor C.  | 422             | 343     | 508             | 15, 16, 17 Sept. | Sept. 24 |
| Queenie S.  | 370             | 451     | 638             | 15, 16, 17 "     | " 26     |
| Helen W.    | 302             | 807     | 802             | 15, 16, 17 "     | Oct. 5   |
| Alberta C.  | 519             | 509     | 593             | 15, 16, 17 "     | " 5      |
| Marie C.    | 663             | 691     | 940             | 21, 22, 23 "     | " 14     |
| Sylvia D.   | 584             | 902     | 1070            | 21, 22, 23 "     | " 11     |
| Lila E.     | 895             | 612     | 1095            | 21, 22, 23 "     | " 21     |
| Evelyn T.   | 1135            | 587     | 1385            | 28, 29, 30 "     | " 22     |
| Gertrude J. | 225             | 289     | 408             | 28, 29, 30 "     | " 18     |
| Mary S.     | 848             | 803     | 1109            | 26, 27, 28 Oct.  | Nov. 23  |
| Lillian S.  | 725             | 637     | 645             | 26, 27, 28 "     | " 5      |
| Average     | 608             | 603     | 836             |                  |          |
| Evelyn W.   | 571             | 484     | 568             | 28, 29, 30 Sept. | Oct. 15  |

Comparing first the horizontal dorsal and the sitting positions, we find that the responses are very close in the 2 groups, the average being 608 cc. and 603 cc. respectively. Taking the dorsal position as a standard of 100%, the sitting position gives a 98% response. When we compare the results of the 2 recumbent postures we find that the average response in the lateral position is 836 cc. or 136%. The spread of individuals runs from 89% to 266%. Calculated on the same basis the results of the same 2 positions in Mrs. J. H. would show 100% and 164%.

No effort was made to follow through pregnancy, or any considerable part of it, the subjects who were observed at the Crittenton Home. There has been reported such a series followed through the latter months of pregnancy among whom were estab-

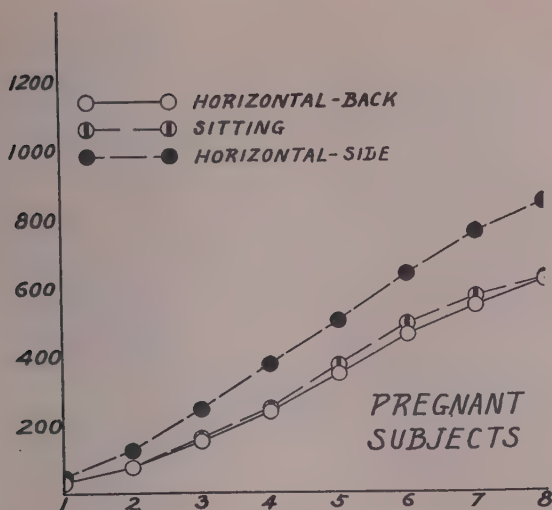


FIG. 2.

Showing summation curves of the Florence Crittenton patients in the various positions of the test.

lished the curve of water diuresis under the conditions of this test. As soon as the results of Mrs. J. H.'s tests were worked out it became evident that the interesting difference in the results of tests in the horizontal lateral position and the others could be checked up by testing a group of patients, as far advanced as possible in pregnancy, with a single series of tests. In this series the Trendelenberg position was omitted because the difference in results was too small to warrant the patient's discomfort.

*Summary.* A series of water diuresis tests have been done on one normal subject throughout pregnancy in various positions. The output was found to be consistently better with the patient in the horizontal lateral position than in any of the others tried. These findings were checked by a single series of tests in 12 normal subjects who were in the last month of pregnancy. In this series the results were similar to the performance of the patient who was followed throughout pregnancy.

## Studies in Kidney Function. II. Effect of Posture of Diuresis.

JAMES C. JANNEY, GERTRUDE RILEY AND ELISABETH W. WALKER.  
(Introduced by A. W. Rowe.)

*From the Evans Memorial, Massachusetts Memorial Hospitals, and the Boston University School of Medicine.*

In the course of studies with the water diuresis test the effect of changes in posture was studied, because of the recorded observation<sup>1</sup> that there is a decided change in urine volume between the horizontal and sitting positions. The postures chosen for this study were the horizontal back, the sitting and the Trendelenberg positions.

Six subjects underwent the whole series of 3 tests each. Six others had done the recumbent and sitting tests in the course of different series of observations. The conditions of the test, however, were identical in the 2 series, with a single exception. In one series the tests were done at weekly intervals, in the other they were done on succeeding days. The method is given in the previous report.<sup>2</sup> Table I shows the results of kidney studies in these subjects by other methods than the diuresis test.

The subjects are classified as were the controls in the previous report,<sup>2</sup> into groups of studied normals and assumed normals. The "studied normal" group (SN) are subjects who have had recent complete physical examinations including extensive kidney tests and who on this basis have been classified as normal. The group of assumed normals (AN) are made up of those who have been normal at previous examinations but where the tests have been so much earlier as to exclude them from the former group. In this group are also put those subjects who had normal kidney tests but who could not be classed as fully normal because they had other defects, which would, however, presumably not affect the renal function. Referring again to Table I, minute traces of sugar and of albumen are not taken as significant as they are generally recognized as being within normal limits. On the above basis 8 of the subjects fall into the SN group and 3 into the AN group.

In the twelfth subject red and white cells were found in the urine sediment. Although the studies of Addis,<sup>3</sup> by detailed sedi-

<sup>1</sup> L'Abbé, Marcel, and Violle, P. L., *Metabolisme de l'Eau*, Paris, Masson et Co, 1927.

<sup>2</sup> Janney, J. C., and Walker, E. W., *J. Am. Med. Assn.*, 1932, **99**, 2078.

<sup>3</sup> Addis, T., *J. Clin. Invest.*, 1925, **2**, 409.

ment counts and computation, show that considerable numbers of both red and white cells are normal, we have classified him as abnormal because of the lack of accurate cell counts. In other respects he was normal.

The experiment was designed to compare the results of the water diuresis test in the horizontal dorsal position, in the sitting position, and in the Trendelenburg position. In the latter the angle was not very high, about 15%. The conditions of the test were the same as those outlined previously.<sup>2</sup> The tests were done at weekly intervals on 6 of the subjects who did all 3 tests. Six other subjects did the tests in the sitting and horizontal back positions in the course of a different study which did not include the Trendelenburg test. In Table II these figures are shown. The average performance of these tests was 1262 cc., which corresponds very closely to the average of all "S" and "A" cases so far studied, which is 1290 cc. The curves are shown in Fig. 1.

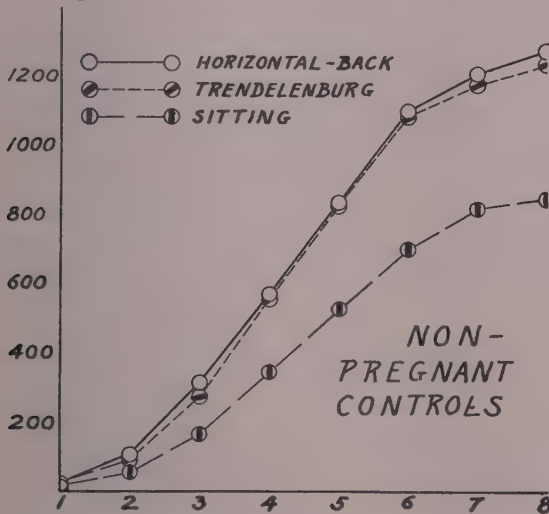


FIG. 1.

Summation curves of the average figures shown in Table II.

Twelve tests were done in the sitting position on 12 different subjects. In all cases there was a cut in urine volume as compared with the individual control reactions of the particular subjects, and also in comparison with the average, of the group. Although there is considerable variation among the individuals the average volume is 823 cc. or 65% of the control figure. Compared to the average normal the output is 64%. It will be noted that the one abnormal sub-

TABLE I. Showing Responses of Subjects to the Investigation of Their Kidney Function.

|                 | Baseline Urine            |                    | Urine Chemistry  |        | N.P.N. | B.U.N. | P.S.P. | B.P. | Mesentherial Differential Diuresis | Diff. |
|-----------------|---------------------------|--------------------|------------------|--------|--------|--------|--------|------|------------------------------------|-------|
|                 | Normal                    | Normal             | Normal           | Normal |        |        |        |      |                                    |       |
| Studied Normals |                           |                    |                  |        |        |        |        |      |                                    |       |
| 1               | Normal                    | Normal             | Normal           | Normal | 39     | 19     | Normal | 102  | 1037                               | 31    |
| 2               | "                         | "                  | "                | "      | 23     | 11     | "      | 62   | 1006                               |       |
|                 |                           |                    |                  |        |        |        |        | 110  | 1029                               | 24    |
| 3               | "                         | "                  | "                | "      | 23     | 11     | "      | 70   | 1005                               |       |
|                 |                           |                    |                  |        |        |        |        | 122  | 1031                               | 29    |
| 4               | "                         | "                  | "                | "      | 27     | 13     | "      | 72   | 1002                               |       |
|                 |                           |                    |                  |        |        |        |        | 110  | 1031                               | 27    |
| 5               | "                         | "                  | "                | "      | 35     | 18     | "      | 70   | 1004                               |       |
|                 |                           |                    |                  |        |        |        |        | 112  | 1020                               | 17    |
| 6               | .43% sugar*               | "                  | "                | "      | 31     | 16     | "      | 76   | 1003                               |       |
|                 |                           |                    |                  |        |        |        |        | 98   | 1027                               | 19    |
| 7               | Normal                    | "                  | "                | "      | 31     | 15     | "      | 68   | 1020                               |       |
|                 |                           |                    |                  |        |        |        |        | 122  | 1032                               | 21    |
| 8               | .42% sugar*               | "                  | "                | "      | 30     | 15     | "      | 78   | 1025                               |       |
|                 |                           |                    |                  |        |        |        |        | 104  | 1030                               | 27    |
| Assumed Normals |                           |                    |                  |        |        |        |        | 60   | 1010                               |       |
| 9               | .33% sugar*               | "                  | "                | "      | 40     | 19     | "      | 106  | 1036                               |       |
|                 |                           |                    |                  |        |        |        |        | 60   | 1031                               | 33    |
| 10              | Alb. S.P.T.,<br>Sug. .31% | "                  | High residual N. | "      | 27     | 13     | "      | 108  | 1035                               |       |
|                 |                           |                    |                  |        |        |        |        | 58   | 1021                               | 29    |
| 11              | .33% sugar*               | "                  | "                | "      | 27     | 14     | "      | 96   | 1037                               |       |
|                 |                           |                    |                  |        |        |        |        | 58   | 1032                               | 25    |
| 12              | Normal                    | Few WBC<br>and BBC | Normal           | "      | 32     | 16     | "      | 112  | 1004                               |       |
|                 |                           |                    |                  |        |        |        |        | 76   | 1032                               | 27    |
|                 |                           |                    |                  |        |        |        |        |      | 1005                               |       |

\*Otherwise normal.

TABLE II.

Responses of Subjects to the Diuresis Test in the Various Positions.

Upper figure in the % columns compares the result with this individual control performance. Lower figure compares with 1262 cc., the average control performance. Under present group control the two numbers at the left represent individual tests and the number at the right the average of these tests.

|                 | Present<br>Group<br>Control | Average<br>Normal | Sitting | %  | Trendelenberg | %   |
|-----------------|-----------------------------|-------------------|---------|----|---------------|-----|
| Studied Normals |                             |                   |         |    |               |     |
|                 | 750                         |                   |         | 76 |               | 107 |
| 1               | 872                         | —                 | 663     | 51 | 928           | 72  |
|                 | 993                         |                   |         | 63 |               | 85  |
| 2               | 1507                        | —                 | 944     | 73 | 1273          | 99  |
|                 | 1484                        |                   |         | 54 |               | 105 |
| 3               | 1249                        | —                 | 676     | 52 | 1304          | 101 |
|                 | 1209                        |                   |         | 63 |               | 121 |
| 4               | 747                         | —                 | 471     | 36 | 903           | 70  |
|                 | 562                         |                   |         | 64 |               | 97  |
| 5               | 1830                        | —                 | 1166    | 90 | 1771          | 136 |
|                 | 1624                        |                   |         | 79 |               |     |
| 6               | 1436                        | —                 | 1134    | 43 | —             | —   |
|                 | 1465                        |                   |         | 55 |               | —   |
| 7               | 1624                        | —                 | 703     | 86 | —             | —   |
|                 | 1075                        |                   |         | 68 |               | —   |
| 8               | 1022                        | —                 | 881     | —  | —             | —   |
|                 | 979                         |                   |         |    |               |     |
| Assumed Normals |                             |                   |         |    |               |     |
| 9               | 1139                        | —                 | 402     | 35 | —             | —   |
|                 |                             |                   |         | 31 |               | —   |
| 10              | —                           | —                 | 1260    | 98 | —             | —   |
|                 | 1282                        |                   |         | 56 |               |     |
| 11              | 1348                        | —                 | 760     | —  | —             | —   |
|                 | 1413                        |                   |         | 59 |               |     |
| Average         |                             |                   |         |    |               |     |
|                 | 1262                        | 1290              | 823     | 65 | 1236          | 98  |
|                 |                             |                   |         | 64 |               | 96  |
| 12              | 1600                        | —                 | 874     | 63 | 1340          | 97  |
|                 | 1385                        |                   |         | 68 |               | 104 |
|                 | 1170                        |                   |         |    |               |     |

ject conforms very well to the performance of the others. The figures for the sitting position and also for the Trendelenberg positions are presented in Table II and in Fig. 1.

The Trendelenberg position test was carried out on 6 subjects only and the volume is almost unchanged from the control figures, a drop

of only 2%, and compared to the general average a drop of 4%. In this test also it will be seen that the single abnormal case conforms well to the performance of the others.

The figures for the test in the sitting position confirm the experience of others and they illustrate well another reason for rest in bed for all cases showing kidney impairment or cardio-renal disease of important extent. Rest in bed is generally recognized as the way to save the heart by cutting down physical exertion. It does this not only by limiting motion and reducing the tissue demand for blood, but also by allowing the heart to do its work at a much lower level of blood pressure than in the upright position. Whereas the average normal blood pressure is 120/80 in the standing position, when the patient is basal it is reduced to 80/50, and even when recumbent without being basal the reduction will approximate about 20/15 points. This not only saves the heart, but it necessitates less capillary and arteriolar constriction and presumably allows more blood flow through the kidneys. Viewed purely from the point of view of a nephropathy without cardiac complications, the better the circulation through the kidney the better opportunity there is for a damaged kidney to do its job.

The close correspondence in output between the horizontal back position and the Trendelenberg position probably depends on the fact that in the latter position the angle of tipping was so low, about 15 degrees. Had it been greater we feel that the results would have shown a figure more closely approaching that found in the sitting position.

*Summary.* It has been shown with a group of normal subjects that the volume response to a water diuresis test is greater in the horizontal than in the sitting posture. The Trendelenberg position was also used and gave values slightly different from those in the horizontal position, and in the expected direction. The theoretical importance of the horizontal position, aside from the saving of effort, has been discussed relative to kidney disease and the toxemias of pregnancy.

## 7145 C

## Further Experiments with B. Pertussis Filtrate.

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Toomey and McClelland<sup>1</sup> have described a substance found in cultures of veal brain broth during the growth of *H. pertussis*. The following is an additional report on the character of this filtrate, termed for convenience B.P.F.

The strain of *B. pertussis* used was number 778 of the American Type Culture Collection, originally isolated by Park. It was planted and grew luxuriantly in 50 Erlenmeyer flasks, each of which contained 50 cc. of bloodless veal brain broth medium.<sup>1</sup> For 50 successive days, the contents of a flask were treated each day as described previously.<sup>1</sup> The filtrate obtained was kept sterile by the addition of 0.2% of tricresol. The 50 specimens collected were divided into groups of 10 each.

One group of filtrates was injected intradermally into the arms and forearms of at least 6 individuals, in doses of 0.1 cc. There was no appreciable change in the toxicity of the filtrate obtained from these growths as evidenced by the reactions to skin tests until the filtrate of the twelfth day was harvested. The filtrates that were obtained after the twelfth day always gave local inflammatory reactions when injected intradermally in the skins of individuals; these reactions were still present 24 hours after the injection, although a positive reaction was sometimes obtained with those filtrates that had grown for from 8 to 12 days. The filtrates obtained after the twentieth day of growth, however, contained no greater amount of B.P.F. than those obtained from the twelfth to the twentieth day.

Cultures were taken of the growths preceding each daily filtration to make certain of purity and to note the length of time that organisms remained viable. The transplantation was made on solid veal brain agar. Beginning with the twentieth day, the transferred growth became more sparse. By the twenty-fifth day, little or none of the transplanted material had grown, and by the thirtieth day, all the organisms were dead since no further growth could be obtained

<sup>1</sup> Toomey, John A., McClelland, Joseph E., *Proc. Soc. Exp. Biol. and Med.*, 1933, **31**, 34.

on subculture. Demonstrable death of the organisms started about the twentieth day.

In the group of patients that was tested as described in the foregoing, the reaction itself and not its character or extent was noted. To test the difference in local reactions quantitatively, B.P.F. was obtained from cultures which grew for 1, 8, 12, 20, 30, 40, and 50 days, respectively. One tenth cc. of each of the 7 filtrates was injected into each of 24 individuals and the reactions were read in 6 and 24 hours. The first injection of B.P.F. obtained from the 24-hour growth frequently caused an immediate but fleeting local reaction which disappeared before 24 hours had elapsed. The reactions obtained after injections of filtrates that were harvested after the eighth and subsequent days were definite 6 hours after injection and were still present after 24 hours. The maximum reaction value was reached about the twelfth day.

In order to study the effect on B.P.F. production, batches of new media were made up with the various factors of the original media, alone or in combination. These solutions were as follows: No. 1. Sodium phosphate, 1.6 gm.; water, 98.4 cc. No. 2. Sodium phosphate, 1.6 gm.; sodium chloride, 1.6 gm.; water, 96.8 cc. No. 3. Sodium phosphate, 1.6 gm.; sodium chloride, 1.6 gm.; peptone, 3.3 gm.; water, 93.5 cc. No. 4. Sodium phosphate, 1.6 gm.; sodium chloride, 1.6 gm.; peptone, 3.3 gm.; veal solution, 93.5 cc. No. 5. Veal solution, 100 cc. No. 6. Sodium phosphate, 1.6 gm.; sodium chloride, 1.6 gm.; peptone, 3.3 gm.; veal brain solution, 93.5 cc.

Culture number 778 was planted in each flask of the 6 media solutions listed and incubated for 20 days. The growth was centrifuged, the supernatant fluid passed through a Mandler N and W filter, and the filtrate solutions from the 6 growths were tested out on 6 individuals with the 1/1 dilution, on 6 with the 1/25 and on 6 with the 1/50 dilution. The amount injected intradermally each time was 0.1 cc. The reactions were read after 6 and 24 hour intervals. There were no reactions to filtrates 1 and 2. Solution number 3 had a fair bacterial growth in the medium and only a moderate production of toxic filtrate as determined by skin tests. Number 5 medium, containing only veal solution, had a bacterial growth and a toxin production similar to number 3. Media combinations 4 and 6 were nearly alike in their growth promoting qualities, although the addition of brain infusion to the latter seemed to enrich it slightly. These conclusions were found by noting the comparative extent and character of the reaction and the length of time it persisted after each intradermal injection in test subjects.

From these experiments, it seems that the addition of veal or brain infusion brings about a better growth of the organisms and an increase in the production of B.P.F. units. The corollary seems to hold true that the production of B.P.F. depends on the healthy growth of the organisms.

Culture number 778 was again planted in Erlenmeyer flasks, each containing 50 cc. of veal brain broth medium. After 20 days of growth, the organisms were separated from the filtrate and the latter was passed through Mandler N and W filters as in other experiments. The organisms remaining at the bottom of the centrifuge tube were killed and the whole was brought up to the original volume with normal saline. This we called B.P. vaccine. The B.P.F. and the killed B.P. vaccine were tested out on 82 individuals. It was found that more people reacted with the B.P.F. than with the B.P. vaccine solution made as described, the local reactions being greater with the filtrate injections in 54 of the 82 cases.

Twelve day B.P.F. units in dilutions of 1/1, 1/10, 1/25 and 1/50 in doses of 0.1 cc. were injected intradermally in 35 babies about 12 months old. After an interval of 6 hours, there were good reactions in practically all instances, the inflammatory reaction sometimes becoming as large as 5/8 of an inch (1.54 cm.) in diameter with induration. After 24 hours these reactions had become negative or had materially lessened in size.

When 23 mothers and their newborn babies were simultaneously tested, the local reactions at the end of 6 and 24 hours were about equal.

Children who had whooping cough in an inactive stage were tested intradermally with 0.1 cc. doses of B.P.F. and usually showed good reactions which persisted for more than 24 hours. When they were tested again on an average of from 6 to 12 days later, an enhancement of the reaction occurred.

Boiling B.P.F. for 10, 30, and 90 minutes did not decrease the reaction value of the mixture.

Culture number 778 was planted in brain veal broth medium to which 20% placental blood was added to determine whether the toxic value of the filtrate would be enhanced by blood. It was found that the number of skin test units obtained with this preparation was not greater than that obtained on veal brain broth medium.

Combinations of equal parts of B.P.F. and convalescent pertussis serum in various dilutions were injected intradermally in humans. There was no consistent neutralization of the toxin; in fact, in

many instances, there was obvious enhancement of the local response.

The striking thing about the growth of *H. pertussis* culture number 778 in liquid veal brain broth media was the formation of a thick tenacious substance. The resemblance between this material and the thick stringy sputum coughed up by children after an attack of whooping cough was obvious.

Culture number 778 was also planted on many types of solid media, 1½% agar with broth, Pelouze's gonococcus and Bordet-Gengou media. On the broth agar and Pelouze's media, the growth was rapid and in about 5 to 6 days the surface began to appear dully glazed. After this time, the growth stuck tenaciously to the medium so that separation was not possible with the ordinary platinum loop. In marked contrast, however, there was never any stickiness to the growth that appeared on the surface of the Bordet-Gengou media.

Growths of culture number 778 on Bordet-Gengou, as well as on veal brain broth media, were blown, dropped and syringed into the nasal passages of 5 *Macacus rhesus* monkeys weighing on an average of 6 pounds. In no instance was there a response that suggested whooping cough, either clinically or hematologically. This may be explained by the fact that culture number 778 was old and probably had lost its pathogenicity.

## 7146 C

### Further Studies on the Specific Carbohydrates of *Vibrio Cholerae* and Related Organisms.\*

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In a previous note<sup>1</sup> on the specific carbohydrates of cholera and cholera-like vibrios some preliminary results were indicated. Since then our work has been considerably broadened, and we are able to extend our conclusions.

The method of extracting the carbohydrates used at first con-

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\* This work was done under the auspices and with the support of the Indian Research Fund Association.

<sup>1</sup> Linton, Richard W., and Shrivastava, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 600.

sisted essentially of coagulating the vibrios by heat in acid solution and then separating the specific substances from the supernatant fluid by alcoholic precipitation. The vibrios were grown on agar, and further study has convinced us that vibrios so grown carry agar along with them in amounts sufficient to lead to considerable confusion, and whose removal is a matter of some difficulty. Organisms of the 2 vibrio types, whose carbohydrates contain galactose and arabinose respectively, were then grown for 5 days in 30 liters of 1% peptone water at pH 8.0, and the 2 characteristic sugars were obtained from this growth. In this method, however, the polysaccharide yield is too small to be of practical use.

We have therefore adopted a method used by Furth and Landsteiner<sup>2</sup> in which the organisms themselves, and not the fluids in which they have been growing, are used as the source of the polysaccharides. The organisms are washed repeatedly in distilled water, and centrifuged, until the supernatant fluid after hydrolysis no longer reduces Benedict's solution. The washed vibrios are dissolved in alkali, precipitated in alkaline solution with 1.5 volumes of alcohol, the precipitate dissolved in water and protein removed by the addition of acid. The polysaccharide in solution is then finally precipitated by alcohol in alkaline solution, washed in alcohol, and dried. The method has the advantages of being more rapid and less expensive than the first one, and of giving a good yield of the polysaccharide.

*Types of Vibrios.* By this method we have now prepared and analyzed the specific carbohydrates of 38 cholera and cholera-like vibrios. These have fallen into 3 groups as follows: 26 vibrios from clinical cholera with galactose-containing polysaccharide. Agglutinable. 8 vibrios from clinical cholera with arabinose-containing polysaccharide. Agglutinable. 4 vibrios from water with arabinose-containing polysaccharide. Non-agglutinable.

All the vibrios in the first group are agglutinable, and 15 of them have been used in the manufacture of cholera vaccines. It is interesting to note that the one "El Tor" vibrio investigated fell into this group. All the arabinose-containing organisms from clinical cholera in the second group are also agglutinable. Four of them have been used in vaccines. The water vibrios are non-agglutinable and are arabinose-containing as in Group 2.

The first and third groups of vibrios were described previously. We must now recognize an intermediate group of vibrios, having the same carbohydrate as the water vibrios and occurring in cholera,

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<sup>2</sup> Furth, J., and Landsteiner, K., *J. Exp. Med.*, 1928, **47**, 171.

and at the same time being "typical" enough in the characteristics usually considered to be placed among the carefully chosen vibrios used in vaccines. The interrelationships between the 3 groups are not yet clear. It appears, however, that the agglutination reaction among the vibrios does not depend upon the presence of arabinose-containing carbohydrate.

*Carbohydrates in "Rice-Water" Stools.* Between one and 2 liters of the typical "rice-water" stool was collected from each of a series of 11 cholera patients. This material was concentrated to 300 or 400 cc. in acid solution on the water bath, the heavy flocculum removed in the centrifuge, and the concentrate treated as in the method of Furth and Landsteiner. The flocculum which appeared after the final alcoholic precipitation in alkaline solution was biuret-negative, and until hydrolyzed did not reduce Benedict's solution. The quantities obtained, while small, enabled us to identify the sugars they contained as the 2 characteristic ones of the cholera vibrios. Seven of the stools yielded galactose-containing and 3 arabinose-containing polysaccharide. In the eleventh stool, taken from a patient who recovered, no definite conclusion as to the nature of the sugar could be drawn.

*Antibactericidal Effect of Specific Polysaccharides.* The identification of the specific polysaccharides of *Vibrio cholera* in the stools of cholera patients gains in interest from several experiments which have been made to demonstrate an antibactericidal effect of the carbohydrate, since bacteriolysis is probably a means of defense of the body against vibrios. Such an experiment is as follows:

TABLE I.  
Antibactericidal Effect of Specific Carbohydrate of Cholera Vibrio No. 79.

| Dilution of<br>24 Hour<br>Culture. |                    |       |        | Serum Dilutions:      |       |        | Control |
|------------------------------------|--------------------|-------|--------|-----------------------|-------|--------|---------|
|                                    | 1:25               | 1:250 | 1:2500 | 1:25                  | 1:250 | 1:2500 |         |
| 10 <sup>s</sup>                    | +                  | +     | +      | 0                     | 0     | 0      | +       |
| 10 <sup>6</sup>                    | +                  | +     | +      | 0                     | 0     | 0      | +       |
|                                    | Carbohydrate added |       |        | No carbohydrate added |       |        |         |

+ = Growth; 0 = No Growth.

Each tube contained 0.5 cc. of fresh guinea pig complement diluted 1:10, 1.0 cc. of bacteriolytic antiserum, and 0.5 cc. of the dilution of organisms. One-half cc. of a 0.1% solution of the specific carbohydrate was added to the first series of tubes, and an equal amount of normal saline to the second. The tubes were incubated for 24 hours, the entire contents then placed in broth and the readings made after a further 24 hours' incubation. The car-

bohydrate solution was derived from the organism used, and contained arabinose. Similar antibacteriolytic power was shown in an experiment in which galactose-containing organisms and carbohydrate were used.

*Conclusions.* The vibrios which have been studied in this endemic area for cholera (Bengal) appear to fall into 3 groups on the basis of their carbohydrate content and agglutinability. In the majority of vibrios derived from cholera, galactose is the characteristic sugar. A second smaller group of vibrios, also from cholera, contain arabinose. These do not appear to differ from the first in agglutinability or in virulence, to judge from the type of case in which they have been found. The third group consists of non-agglutinable vibrios derived from water, some of which differ from *Vibrio cholerae* only in their failure to react with cholera agglutinating serum. All of these are arabinose-containing vibrios.

Polysaccharides containing both arabinose and galactose have been isolated from "rice-water" stools of cholera cases.

Both types of carbohydrate have an antibacteriolytic effect.

## 7147 P

### Experimental Poliomyelitis. Active Immunization with Neutralized Mixtures of Virus and Serum.\*

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Work on the nature of virus-antiserum combinations has indicated that although the virus in such mixtures is completely inactivated, the effect on the virus is not a destructive one, but more in the nature of a "neutralization" phenomenon. It is possible to recover active virus from neutral virus-serum unions by such means as dilution, and cataphoresis.<sup>1-5</sup> It is therefore not surprising that

\* This work is supported by public and private subscription.

<sup>1</sup> Andrews, C. H., *J. Path. and Bact.*, 1928, **31**, 671.

<sup>2</sup> Douglas, S. R., and Smith, W., *Brit. J. Exp. Path.*, 1928, **9**, 213.

<sup>3</sup> Long, Perrin H., and Olitsky, Peter K., *J. Exp. Med.*, 1930, **51**, 209.

<sup>4</sup> Olitsky, Peter K., and Long, Perrin H., *J. Exp. Med.*, 1929, **50**, 263.

<sup>5</sup> Olitsky, Peter K., Rhoads, C. P., and Long, Perrin H., *J. Exp. Med.*, 1929, **50**, 273.

mixtures of virus neutralized by serum have been shown to possess antigenic properties.<sup>6, 7, 8</sup>

Utilizing these facts, with a view toward ultimate development of a safe and practical method for active immunization against poliomyelitis, applicable to humans, investigations were undertaken to determine the conditions under which a high incidence of immunity could be obtained in the experimental animal.

To that end, 2 series of animals, whose serums had previously been tested by the neutralization test to rule out the presence of neutralizing substance, were inoculated subcutaneously and intramuscularly, with neutralized mixtures. In the first series, varying proportions of virus and serum, the potencies of which were only roughly approximated, were used. The second series of animals received inoculations of accurately neutralized mixtures based on careful titrations of virus and serum. Two such balanced mixtures of serum and virus were used in this series; one containing 16 cc. of 5% virus suspension to 1 cc. of human convalescent serum, the other mixture containing 20 cc. of virus to 1 cc. of serum.

Since the purpose of this attempt at immunization was to determine the number of animals that would give a demonstrable response following injection of neutralized mixtures of virus and serum of known titre, the neutralization test was selected as the most desirable criterion for immunity. This is in accord with conclusions reached by Stewart and Rhoads<sup>9</sup> and by Brodie and Goldbloom,<sup>10</sup> that the neutralization test (using small doses of virus) is a more sensitive test for immunity than is the intracerebral test.

All monkeys were bled 4 to 6 weeks following the last inoculation, and the serums tested for the presence of immune substance by the neutralization test. The serums of the second series (12 animals) were tested against 10 infective doses of the titrated virus.

The results of these experiments are summarized in Table I.

The results indicate that a high incidence of immunity ( $\frac{3}{4}$  of the animals treated), as indicated by the neutralization test, follows inoculations with balanced neutral mixtures of serum and virus.

Experiments are now being conducted to determine the degree of this immune response and to what extent this can be developed.

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<sup>6</sup> Andrews, C. H., *J. Path. and Bact.*, 1929, **32**, 265.

<sup>7</sup> Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 185.

<sup>8</sup> Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 115.

<sup>9</sup> Stewart, F. W., and Rhoads, C. P., *J. Exp. Med.*, 1927, **49**, 959.

<sup>10</sup> Brodie, Maurice, and Goldbloom, A., *J. Exp. Med.*, 1931, **53**, 885.

TABLE I.

| Series No. | No. monkeys | Inoculum virus serum | Tot. amt. inoculated, cc. | No. inoculations | Interval between inoculations, days | Results of neutralization tests on serums of treated monkeys                               |
|------------|-------------|----------------------|---------------------------|------------------|-------------------------------------|--------------------------------------------------------------------------------------------|
| 1          | 6           | 1:1                  | 5                         | 1                |                                     | .5 cc. serums of 2 monkeys neutralized .5 cc. of a 5% virus suspension.                    |
|            |             | 3:1                  | 5                         | 1                | *                                   | Serums of 3 monkeys failed to neutralize.                                                  |
|            |             | 4:1                  | 10                        | 1                |                                     | One animal died of tuberculosis.                                                           |
|            |             | 5:1                  | 12                        | 1                |                                     |                                                                                            |
| 2†         | 3           | 16:1                 | 30                        | 4                | 7                                   | .5 cc. serums of 2 of 3 monkeys failed to neutralize 10 infective doses of titrated virus. |
|            | 3           | 16:1                 | 60                        | 7                | 7                                   |                                                                                            |
|            | 3           | 20:1                 | 30                        | 4                | 7                                   |                                                                                            |
|            | 3           | 20:1                 | 69                        | 7                | 7                                   | .5 cc. serums of 3 monkeys neutralized at least 10 infective doses of titrated virus.      |

\* Each animal received the 4 inoculations at weekly intervals.

† The potency of the serum and virus used in this series was determined by titration and was kept constant throughout the experimental period.

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